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The Utility of Sampling and Analysis for Compliance Monitoring of the Biological Weapons Convention

Jonathan B. Tucker, Editor

Proceedings of a workshop held in Washington, DC
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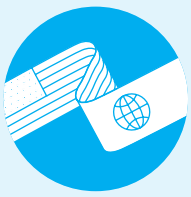

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Contents

Tables

Introduction

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1

On October 7–8, 1996, the Center for Nonproliferation Studies at the Monterey Institute of International Studies and the Center for Global Security Research (CGSR) at the Lawrence Livermore National Laboratory jointly sponsored a *Workshop on the Utility of Sampling and Analysis for Compliance Monitoring of the Biological Weapons Convention*, which was held at the Carnegie Endowment for International Peace in Washington, DC. This workshop was attended by some 40 invited experts from the technical, policy, and industry communities with an interest in biological arms control; these experts included representatives from Canada and the United Kingdom. The purpose of the meeting was to discuss the possible use of biological sampling and analysis for monitoring compliance with the Biological Weapons Convention (BWC), with the aim of generating some useful findings and recommendations.

Background

The BWC was opened for signature in 1972 and entered into force in 1975. This landmark treaty was the first multilateral accord to ban an entire category of weapons of mass destruction. During the quarter-century of its existence, however, the effectiveness of the BWC has been repeatedly undermined by its lack of verification measures, leaving it poorly equipped to deal with a series of alleged treaty violations.

At the time the treaty was negotiated, biological and toxin weapons were generally considered to have little military utility compared with other weapons of mass destruction. Indeed, the United States decided unilaterally to renounce the possession of microbial BW agents in 1969 and toxins in 1970. The perceived lack of a strong military incentive for countries to acquire these weapons reduced the need for a verification regime. At the time, it was also generally recognized that the highly intrusive verification provisions, such as on-site inspection, required to verify the nonproduction of biological and toxin warfare (BTW) agents were anathema to the Soviet Union and other states.

In recent years, both assumptions have changed fundamentally. First, the biotechnology revolution has opened up new and disturbing prospects for the development and mass-production of naturally occurring—as well as genetically modified—BTW agents, potentially increasing the military utility of

these weapons. The number of states suspected of possessing or pursuing a biological and toxin warfare capability has also tripled since the BWC was signed, from four to roughly a dozen.

Second, the perception of arms control verification measures has changed markedly since the BWC was signed. For much of the Cold War, the Soviet Union believed that national security required extreme military secrecy and viewed on-site inspections as tantamount to foreign espionage. Since the late 1980s, however, Moscow has undergone a dramatic conversion and now agrees with the United States that one must "trust but verify." At the same time, the end of the Cold War revitalized multilateral approaches to international security and breathed new life into long-quiescent arms control negotiations.

In the fall of 1992, the Conference on Disarmament in Geneva put the finishing touches on the Chemical Weapons Convention (CWC), a treaty banning the development, production, stockpiling, transfer, and use of chemical weapons. In contrast to the BWC, the chemical treaty contains the most extensive and intrusive verification regime ever negotiated. The current relatively cooperative period of international relations offers a window of opportunity for strengthening the BWC. If this historical chance is lost or squandered, it may not recur for a long time.

History of Efforts To Strengthen the BWC

The current effort to strengthen the BWC is the culmination of a process that began with the Second Review Conference of the treaty in 1986. At that time, US allegations that the Soviet Union was violating the BWC—including a suspicious outbreak of anthrax in the city of Sverdlovsk in April 1979, and claims in the late 1970s and early 1980s that the Soviet Union and its allies were employing toxin weapons ("yellow rain") in Southeast Asia and Afghanistan—raised doubts about the effectiveness of the convention. Because of the lack of verification measures, however, there was no effective way to assess the accuracy either of the US allegations or the Soviet denials.

In an attempt to strengthen the treaty regime, the Second Review Conference adopted a number of politically binding confidence-building measures (CBMs), such as reporting on unusual outbreaks of disease. Unfortunately, less than half of the BWC states parties have submitted the data required under the CBMs on an annual basis. The Third Review Conference in 1991, recognizing both the value of CBMs and their limitations, moved to improve and expand them. At the same time, a number of countries advocated the negotiation of a verification protocol to the BWC. The United States, noting the ease with which BTW agents could be produced with dual-use equipment in small, clandestine facilities, and the level of intrusiveness needed to distinguish reliably between treaty-permitted and treaty-prohibited activities, expressed skepticism that the BWC could be made verifiable.

Despite these reservations, the Third Review Conference agreed to establish an Ad Hoc Group of Governmental Experts To Identify and Examine Potential Verification Measures from a Scientific and Technical Standpoint. This group, which soon adopted the short name "VEREX," met four times over the following year and a half. During this time, the group identified, examined, and evaluated 21 potential verification measures, both on-site and off-site, and also assessed a number of these measures in combination. The VEREX group's final report, issued in September 1993, concluded that although no single measure could distinguish conclusively between treaty-prohibited and treaty-permitted activities, the use of different measures in combination could strengthen the BWC regime and reduce ambiguities about compliance.

VEREX Assessment of Sampling and Analysis

With respect to biological sampling and analysis, the VEREX group identified a number of proven technologies for detecting and identifying pathogenic microorganisms and toxins with a high degree of sensitivity and specificity. The group's final report concluded that sampling and identification "can provide key information of significant quality and quantity, in particular because of the possibility of obtaining an independent confirmation of analytical results in the event that findings are disputed."[1]

The VEREX group noted that the probability of ambiguous results (e.g., false-positive or false-negative) would be reduced if more than one analytical technique and several samples from the same site were used, and if reference data on the microbiological profile of the site environment were taken into account. Nevertheless, the group also identified some limitations associated with sampling and analysis. For example, a negative test result would not necessarily rule out prohibited activities and hence might not resolve all cases of noncompliance ambiguities. Conversely, if a pathogen or toxin were detected during compliance monitoring, it would still be necessary to assess whether the activity associated with the detected material was legitimate or not. Merely identifying a putative BTW agent would not prove a violation of the convention—in the absence, at least, of a "smoking gun" such as munitions filled with biological agents. The VEREX group also noted the rapidity with which biological and toxin agents could be destroyed by sterilization. Although even a thorough clean-up of a production facility could leave behind DNA fragments and other residues that might hint at illicit activities, there would be no means of quantifying the destroyed stocks or of determining whether the "types and quantities" of agents produced were consistent with activities permitted under the treaty.[2]

Establishment of the Ad Hoc Group

In September 1994, a Special Conference of States Parties to the BWC met to consider the VEREX Final Report. The conference then established a new Ad Hoc Group of all interested BWC states parties with the mandate "to consider appropriate measures, including possible verification measures, and draft proposals to strengthen the Convention, to be included, as appropriate, in a legally binding instrument, to be submitted for the consideration of the States Parties."[3]

Since January 1995, the Ad Hoc Group has met periodically in Geneva to pursue the negotiations. Progress has been slow, however, and the group was forced to abandon its initial goal of presenting a draft protocol at the Fourth Review Conference in November 1996. With respect to sampling and analysis, most of the participating states view this measure as a potentially valuable component of a compliance-monitoring regime, but one that poses complex challenges with respect to the significance of analytical findings and the safeguarding of legitimate industrial trade secrets and national security information unrelated to the BWC.

The workshop presentations and discussions in this volume address the complex technical and policy issues associated with the potential use of sampling and analysis technologies for BWC compliance monitoring. Edited versions of the presentations are provided, along with a summary of the discussions, which were conducted on a not-for-attribution basis.

References

1 Ad Hoc Group of Governmental Experts To Identify and Examine Potential Verification Measures from a Scientific and Technical Standpoint, *Summary Report*, Document No. BWC/CONF.III/VEREX/8, Geneva, September 24, 1993, p. 18.

2 Ad Hoc Group of Government Experts To Identify and Examine Potential Verification Measures From a Scientific and Technical Standpoint, "Evaluation [of] Sampling and Identification (On-Site)," Document No. BWC/CONF.III/VEREX.WP.168.

3 Special Conference of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, *Final Report*, Geneva, September 19–30, 1994, Document No. BWC/SPCONF/1, p. 10.

Technologies for Biological Sampling and Analysis

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2

Several sophisticated methods for identifying biological and toxin warfare agents could be employed in a compliance-monitoring regime for the BWC. These techniques generally target species-specific molecules or characteristics, but some can identify broader categories of agents. This discussion focuses on analytical techniques that are currently in active use, although a few newer technologies are also mentioned.

In assessing the strengths and limitations of various methods for identifying microbial and toxin agents, the concepts of *specificity* and *sensitivity* are useful. An analysis method is said to be *specific* if it identifies only the species of the microorganism tested for. If the analysis accurately identifies the species in question, the result is said to be a *true positive*. If the analysis falsely identifies the species—that is, indicates it is present when it is not—the result is said to be a *false positive*. If the analysis fails to identify the species when it is absent, the result is said to be a *true negative*. If the analysis fails to identify the species when it is indeed present, the result is said to be a *false negative*.

Sensitivity refers to the detection limit of an analytic technique and is a relative concept. For our purposes, *high sensitivity* is defined as the ability of an analytic method to detect as few microorganisms as are required for the purpose at hand. If the method is incapable of detecting a sufficiently low number of microorganisms, it is described as having *low sensitivity*. Methods that have too low sensitivity may yield false negatives by failing to detect the residues of BW agents in a cleaned-up facility. Conversely, methods that have extremely high sensitivities may produce false positives by detecting BW microorganisms (such as anthrax) that are naturally present in the environment in trace amounts.

Three analytical methods are currently employed to detect putative biological and toxin warfare agents: (1) *classical biological assay* techniques, involving the culture and testing of live microorganisms; (2) *immunological assay* techniques, based on the antigenic (antibody-inducing) properties of a microorganism or toxin that permit its specific identification; and (3) *genetic analysis* techniques, based on the structure of the microbial DNA. These three methods are discussed briefly below.

Classical Bioassay Techniques

Bioassays involve the culture of viable microorganisms followed by morphological,

physiological, and biochemical tests to identify the microbial species, sometimes augmented with molecular or immunological tests to determine the specific strain. Bacteria can be identified by culturing them under various nutritional or other environmental conditions, while viral agents can be identified with plaque assays, which determine the ability of a live virus to kill cultured animal cells. Animal virulence testing, which determines morbidity and mortality in experimental animals, is the most direct means of determining the pathogenicity of a suspected BW agent. This technique could be employed, for example, to differentiate between a virulent virus developed as a warfare agent and an attenuated viral strain developed for legitimate vaccine production.

Bioassay techniques are highly reliable and have been used for many years in medical diagnostics. One advantage is that they permit an "open-minded" approach: one does not need to know in advance which microorganism one is looking for. Bioassays can also distinguish between viable and nonviable cells. Some methods are partly automated, improving their speed and objectivity.

Nevertheless, classical bioassay techniques have a number of drawbacks. First, they are time-consuming, labor-intensive, and involve growing live infectious material that may be hazardous and require containment. Second, the sampling, shipping, and culture procedures must maintain the viability of the specific microbial agent(s) in the sample. Elevated temperature, exposure to air, and a variety of other factors may kill certain microorganisms. Microorganisms may also be fastidious, requiring highly specific nutrients and physical conditions for reproduction, so one must know the probable identity of the sampled agent in order to select the appropriate culture medium and conditions that meet its physiological needs. Because microorganisms often grow slowly in the laboratory, it may take days or weeks to obtain cultures for identification. Some microbes have yet to be cultured under laboratory conditions, so identifying them requires alternative methodologies.

A third drawback of bioassays is that the quantification of results is problematic, although one can use dilution methods to estimate the original concentration of agent. Finally, companies that work with proprietary microorganisms may be unwilling to provide live samples for analysis, for fear of compromising valuable commercial proprietary information (CPI). Despite these drawbacks, the high reliability of bioassay methods warrants their use under special circumstances—for example, when there is strong suspicion of a potential BWC violation or when investigating unusual outbreaks of disease.

Immunological Assay Techniques

Immunoassays employ antibodies to detect unique protein or nonprotein molecules on the surface of target microorganisms, as well as protein toxins. Immunoassays are well suited for BWC monitoring purposes because they are portable, can be performed rapidly, and can identify dead microorganisms or denatured (inactivated) proteins.

The most sensitive immunoassays employ "monoclonal" antibodies produced by the offspring of a single antibody-producing cell that has been fused with an immortalized (cancer) cell, resulting in a clone of genetically identical cells that produce large quantities of one type of specific antibody. Monoclonal antibodies have very high affinity for their target antigens, and may be able to detect fewer than 500 microorganisms. The enzyme-linked immunosorbent assay (ELISA) detects the antibody-antigen reaction through a fluorescent byproduct, resulting in a ten-fold increase in sensitivity. The detection limit can be reduced further by working in smaller volumes, which increase the concentration of antibody. The concept of linking together two antibodies with different specificities can also increase sensitivity to the point that small numbers of microorganisms could be theoretically detected, although less sensitivity is obtained in

practice because of interference between the binding sites of the two antigens and other technical factors.

A drawback of monoclonal antibodies is that they generally recognize only a single site, or *epitope*, on the antigen. As a result, the technique may not detect natural variants or strains of a microorganism that do not display the target epitopes on the cell surface. One way around this problem is to prepare a "cocktail" of various monoclonal antibodies specific to different proteins on the surface of a microbial agent. Indeed, the BWC inspectorate would probably employ a battery of different antibodies directed at several protein and nonprotein targets to attain high specificity and to complicate the task of evading detection by modifying the microbial DNA.

The immunoassay technique can also identify denatured proteins that may remain after autoclaving (sterilization with superheated steam) or mild chemical treatments. Because antibodies can be produced to recognize the denatured form of proteins, immunoassay may still be effective as long as the proteins are not highly degraded.

Genetic Analysis

The hereditary material of every living organism resides in the sequence of chemical bases contained in two types of nucleic acids, DNA and RNA. Four bases—symbolized by the letters A, T, C, and G—make up the DNA molecule. The four letters are combined into three-letter words, or "codons," which specify the 20 amino-acid subunits of proteins; the relationship between codons and amino acids is known as the genetic code. Most bacteria and viruses contain sections of DNA that have unique sequences of bases. (Although some viruses use RNA as their hereditary material, the same principles apply.) Once the unique sequences in a microorganism's DNA have been determined, they can be detected in samples by means of short sequences of synthetic DNA known as *DNA probes*, which pair up spontaneously with the complementary sequences in the microbial DNA.

Each species of BW microorganism has DNA sequences that are unique to it. The DNA that makes up the full complement of hereditary material (genome) in the average bacterium is roughly 4 million bases long, whereas viral genomes are approximately 100 times smaller. Because DNA probes are each about 15 to 150 bases long, hundreds to thousands of microbial DNA sequences could potentially serve for identification. For this reason, it should be possible to prepare dozens of DNA probes that can identify unique DNA sequences in each species of putative BW agent.

Current DNA probe methods are rapid, requiring less than an hour to complete an identification, and are portable, an important feature for on-site analyses. DNA probes can also identify dead microorganisms, making it easier to protect CPI. Residues from dead microorganisms have even been detected by DNA probes after routine sterilization procedures such as autoclaving.

DNA amplification techniques. DNA probes are often used in conjunction with a powerful DNA amplification technique called the polymerase chain reaction (PCR). This method can copy a particular DNA sequence a million-fold or more, generating enough material so that DNA probes can identify trace quantities of a microorganism present in a sample—as few as tens or hundreds of cells—without the need to grow them into larger colonies over a period of days or weeks. Because PCR reagents are available in kit form, this technique has greatly speeded the diagnosis of infectious diseases, including putative BW agents such as anthrax bacteria. Current PCR techniques can be performed in 10 minutes or less, making it possible to screen rapidly for potential BW agents. Another advantage of PCR is that it can detect nonviable microorganisms, including killed bacteria in autoclaved samples.[1]

PCR is about 10 million times more sensitive than routine culture techniques at

detecting small numbers of microbial agents. In several studies, PCR has been used to detect BW agents such as *Yersinia pestis*, the cause of bubonic plague.[2] In one study, 10 plague bacilli were detected in flea tissue.[3] For *Bacillus anthracis*, the causative agent of anthrax, 10 spores per 100 grams of soil have been detected.[4] In another study involving *Bacillus anthracis*, 100 spores were detected when no attempt was made to extract DNA from the spores, but as few as 2 spores were detected when the DNA was released by mechanical disruption of the cells prior to analysis.[5] Another improved method, known as "immuno-PCR," is a hybrid of immunoassay and PCR. In this technique, one performs an immunoassay, tags the bound product with DNA, and then amplifies the DNA sequence with PCR. This approach makes it possible to detect as few as 500 bacteria.[6]

Despite these impressive results, DNA probes and PCR have a number of limitations. First, the use of this technique to detect microbial pathogens requires the determination of target DNA sequences that are unique to the agents in question, followed by the synthesis of an appropriate set of DNA probes and standards for those targets. This task is labor intensive and could take a number of years, particularly if the probes must be validated to the good manufacturing practice (GMP) standards required by the Western pharmaceutical industry.* Second, the technique is generally most suitable for identifying known microorganisms, because one must decide in advance which DNA sequences to use as probes. Although some primers are available for generic "classes"of agents, such as broad families of bacteria, specificity may then become an issue. Third, DNA probes can detect specific DNA sequences but do not prove the infectivity or pathogenicity of a microbial agent, which usually requires live-animal testing.

The sensitivity and specificity of PCR depend on both the length of the target DNA sequence and the length of the PCR "primers," which bind to the target DNA to initiate the amplification process. As one tests for shorter microbial DNA sequences (e.g., 100 rather than 500 to 1,000 DNA base-pairs), the sensitivity of the technique increases but its specificity may decline, because it is possible that several different microbial species may have identical or nearly identical short DNA sequences in the genetic region selected. Moreover, because changes in the host DNA sequence that serves as the target for the PCR primers can impair amplification and gene-probe identification, the method might fail to detect a novel strain whose DNA sequence differs from that of the standard agent.

The appropriate "stringency," or specificity, of a PCR analysis can be determined by the choice of DNA primer, as well as by the reaction conditions. For this reason, standardization of conditions and careful selection of primer sets are important. High stringency refers to the ability of the PCR primer to bind exclusively to its complementary sequence in the microbial DNA, whereas low-stringency primers can bind to DNA sequences with some mismatches. Thus, whereas long primers are generally the most specific, they may detect only one strain of a BW agent while excluding related strains.

Primers can often be devised that allow one to detect a larger set of closely related microorganisms.[7] For this reason, two levels of detection have been proposed, based on the characteristics of the DNA probe. The first level would use a probe with fairly broad specificity to identify the species of pathogenic bacteria or viruses to which a suspect agent belongs by detecting a DNA sequence common to all strains in that group. The second level then would provide strain-specific identification by using longer, high-stringency

* Good manufacturing practice (GMP) refers to a detailed set of procedures designed to ensure reliability, reproducibility, and quality-assurance in the manufacture of biopharmaceutical products such as vaccines and antibiotics. GMP also involves extensive documentation of manufacturing processes.

probes specific to each strain of microorganism targeted for detection.[8]

Genetic fingerprinting. Another kind of genetic analysis is known as restriction-enzyme fragment length polymorphism (RFLP) analysis, or "genetic fingerprinting." This technique involves the use of special "restriction" enzymes that cut microbial DNA at specific sites. Such treatment results in a pattern of DNA fragments of different sizes, which can be analyzed by separating the fragments on a gel, resulting in a characteristic pattern of bands. Because different DNA sequences result in distinct patterns of bands, such maps reveal the extent to which two strains of a bacterium or virus differ genetically.

All microbial pathogens can be "fingerprinted" in this way by analyzing their genetic material. (Because genetic fingerprinting requires relatively large quantities of DNA, trace amounts of genetic material can be amplified for such analysis using PCR.) Those viruses that use RNA as their genetic material can be subjected to fingerprint analysis after first "reverse-transcribing" the RNA into a DNA copy. For many microbial pathogens, scientists have compiled a library of characterized strains that can be compared with a newly discovered strain, so that genetic fingerprinting often provides enough information to determine the source of a virus and whether it has been modified genetically in the laboratory. Because minor genetic differences always exist among the various strains of a pathogenic microbe, it is likely that a laboratory-developed strain would be genetically distinct from an indigenous strain. Moreover, an indigenous strain that has been produced in large quantities is likely to be more genetically homogeneous than the causative agent of a natural epidemic.

Nevertheless, genetic fingerprinting has some serious drawbacks for BWC compliance monitoring. The fact that it is easy to change the band pattern by modifying only a few DNA bases could result in false negatives. For this reason, this technique might be evaded fairly easily by a determined cheater and thus is probably not ideal for pursuing alleged violations of the BWC. Genetic fingerprinting could, however, serve to verify the declared production of specific microorganisms for nonprohibited purposes. It would also be a valuable technique for investigating the alleged use of biological weapons or unusual outbreaks of infectious disease.

Other Analytical Methods

A few other analytical methods offer some promise for BWC compliance monitoring. For example, microorganisms contain several families of molecules, such as fatty acids and oligosaccharides, that are heterogeneous with respect to size or molecular weight. Because the ratios of these molecules are characteristic of each microbial species, it should be possible to identify them with high specificity. These molecules can also survive autoclaving and other common sterilization procedures, and the molecular ratios are difficult or impossible to disguise in an effort to evade detection.

Gas-liquid chromatography (GLC) profiles of long-chain fatty acids from cell membranes have been used successfully in clinical microbiology laboratories and could fit the needs of a BWC compliance-monitoring regime. Microbial species and even strains can be differentiated and identified on the basis of their fatty-acid GLC profiles. Isolation of fatty acids from dead microorganisms is straightforward, a typical analysis takes only two hours, and the equipment can be made portable. Because the number of microbes required for analysis is larger than that needed for immunoassays, and standardized growth conditions are often required, GLC may not be well suited for analysis of environmental samples. Instead, the technique would be more useful for analyzing bacterial seed stocks or samples obtained from production fermentors.[9]

Physiochemical analysis techniques, such as spectroscopy, may be useful for identifying biological or toxin agents in particular situations and may be improved in the future to the point that they could become standard methods for BWC compliance monitoring. For example, gas chromatography/mass spectrometry (GC/MS) is widely utilized in the chemical industry and could be employed to identify nonprotein or protein toxins. Mass spectrometry is finding increasing use for biological identification, and it is likely to advance rapidly.[10] Other spectroscopic methods, such as nuclear magnetic resonance (NMR) or Fourier-transform infrared (FTIR) spectroscopy, are not yet sufficiently developed to be useful for biological detection or identification purposes, but have considerable potential. In the forseeable future, new methods will become available that offer increased power and simplicity, such as arrays of DNA probes immobilized on silicon or glass chips for gene sequencing or detection.[11]

Conclusions

A number of sampling and analysis techniques are potentially suitable for BWC compliance monitoring, although each has its strengths and weaknesses. Since no analytical method is perfect, false positives and false negatives are always possible outcomes. One consequence of this fact for a BWC compliance regime is that a positive result obtained with a single analytic method must be treated with caution, since it could be a false positive. For this reason, the results of a test should corroborated with at least one other "orthogonal," or independent, analytical method based on different scientific principles.

Using at least two independent analytical techniques for BWC compliance monitoring makes it unlikely that the same types of systematic errors will arise with each method, reducing the risk of false negatives or false positives to an acceptable level. In order to interpret the significance of the analytic results for BWC compliance, however, evidence of violations obtained by sampling and analysis must not be viewed in isolation but interpreted in the light of data from other sources. The inspection team must also give the inspected site an opportunity to respond to observed anomalies, since a false allegation could cause serious damage to the reputation of an innocent facility.

Discussion

Sampling versus analysis. An industry representative noted that sampling is a separate activity from analytical testing, with its own set of difficulties and risks. Industry has particular concerns over what, where, when, how, by whom, and under what circumstances samples are taken. In the future, more discussion should focus on the issue of sampling procedures. Another participant noted that the three main analytical techniques discussed would be incapable of detecting previously unknown toxins or microbial pathogens, which are occasionally discovered. This weakness is of serious concern because emerging infectious agents and toxins may be known in some countries but not in others.

Orthogonal testing. An industry representative noted that the medical diagnostics industry makes use of multiple confirmatory tests to reduce the incidence of false positives. For PCR assays, a confirmed positive requires positive results from two different reactions using different primer pairs to different genomic sequences. Further confirmation can be obtained by sequencing the PCR products. For HIV testing, in which a false positive could be devastating to the patient, a positive PCR result must be confirmed by a positive immunoassay result. In other cases, two different immunoassays that measure unique protein targets are used. One reservation concerns immuno-PCR, which has not been validated for routine diagnostic testing owing to its high false-

positive rate. In its current form, this technology may not be appropriate for BWC compliance monitoring.

Role of classical biological assays. Several participants expressed support for the use of traditional bioassays, noting that public-health organizations such as the US Centers for Disease Control accept the results of immunoassays or PCR only if they are confirmed by bioassays. One reason is that culture methods cannot be disputed and are capable of distinguishing viable samples from nonviable ones. Bioassay methods are also generally available at plant sites, so their on-site use might be more palatable from an industry perspective. The chief drawback of bioassays is that they cannot detect nonviable microorganisms.

Comparison with chemical analysis. Differences between sampling under the BWC and the CWC were discussed. Whereas the characterization of biological samples is more complex than that of chemical samples, there are a number of similarities. Whether a given facility is making a chemical or a biological product, it must have the means to do quality-assurance testing on its own products, so the appropriate analytical methods are available on site. These methods may not necessarily be capable of characterizing biological materials to the degree required for BWC compliance monitoring, but they should at least provide a starting point for further analysis.

Many operational aspects of sampling and analysis under the BWC and CWC will be quite similar. The main problem, of course, is the need to protect manufacturing processes and other sensitive trade secrets. In chemical manufacturing, the process usually determines the product. In the biotechnology industry, however, the product itself may have proprietary characteristics, such as a particular DNA sequence or microbial strain. Because it is possible to reverse-engineer a genetically engineered microorganism, the biopharmaceutical industry considers specific information about products as well as production processes to be of proprietary concern.

Microbial strain analysis. Participants agreed that in order to determine whether an inspected facility is working on a vaccine or a BW agent, one would have to analyze down to the level of specific microbial strains rather than species. For example, viral strains used for the production of live vaccines are attenuated to make them less pathogenic, whereas strains developed as BW agents are selected for enhanced virulence and infectivity. Participants also noted the need to build internal controls into assays to avoid false-positives arising from inadvertent contamination, and to take a sufficient number of samples at a given site to ensure statistically significant results.

One participant suggested that an expanded database of microbial DNA sequences would assist in determining the geographic and temporal origin of sampled microorganisms. Such a phylogenetic database would be particularly useful for investigating the alleged use of biological weapons, or accidental releases such as the 1979 Sverdlovsk anthrax outbreak, by providing background data on which microbial species and strains occur naturally in a particular area. Although a clever proliferator who sought to maintain deniability might use a local strain of microorganism, this would not necessarily be the case. DNA sequences of related microorganisms are currently available for some microbial species but not for others. Although the use of computers to reconstruct the phylogeny of microorganisms has made major strides in the last ten years, existing methods still rely on relatively simplistic models of the microevolutionary process. Accordingly, more attention should be given not only to the biochemical and genetic side, but also to

the development of improved computer algorithms.

Good manufacturing practice. The discussion turned to the question of GMP and its potential utility in a verification protocol. It was noted that one of the beneficial characteristics of GMP is to make it harder—but not impossible—to use a production line for nonapproved purposes. Only Western companies really operate under a GMP regime, however, and countries most likely to be BW proliferants are not likely to follow such stringent manufacturing standards. Another participant noted that even in the United States, GMP is not applied across the full spectrum of biological production activities. Veterinary biologicals, for example, are not produced under a GMP system. In the European Union, specific GMP standards vary widely among countries. This variation could be confusing to an inspection regime that is geared toward a GMP-like facility. One participant noted that the British government does not consider GMP to be a useful indicator. GMP also presents problems from the standpoint of confidentiality, because industrial facilities would be reluctant to share all of their GMP package with inspectors.

Analysis of degraded samples. Dr. Morse was asked about the analysis of degraded or denatured samples. He responded that the ability to detect a degraded sample, for example an autoclaved sample or waste product, depends on the target of the analysis. DNA, for example, is often quite stable, and DNA probe/PCR methods have detected microbial DNA in autoclaved samples. In the case of highly infectious or virulent microorganisms, analysis of killed specimens is desirable for safety reasons. A paper written a few years ago described the detection of tuberculosis bacteria in autoclaved samples at levels of a few thousand organisms per sample.

Detection of RNA in degraded samples is somewhat more difficult. To detect the expression of a gene (e.g., to determine whether a viable or metabolizing microorganism is present in a sample), one could analyze for messenger RNA, which indicates that the gene has been expressed. One could also perform a variant of PCR to amplify the microbial RNA. The disadvantage of this approach is that because RNA molecules are less stable than DNA, they would be destroyed by many of the same treatments that kill microorganisms, making RNA harder to detect. Finally, the ability to detect proteins (such as protein toxins) with immunoassay in a degraded sample varies considerably depending on the method of inactivation. Many proteins are denatured but not destroyed by autoclaving.

Need for validated assays. An industry representative noted the lack of data relating to the use of laboratory assays in field situations. With respect to the false-positive rate, there is little evidence to support the assertion that analytical techniques designed to identify a specific BW agent (such as *Bacillus anthracis*) will not react with thousands of other microorganisms that may be present on an industrial site, some of which are phylogenetically related to BW agents (e.g., other species of bacilli). The questioner also took issue with Dr. Morse's statement that assays could be performed on-site with degraded samples, as would be the case if the production system was cleaned in advance of an inspection. In fact, there has been little practical field experience with analyzing degraded samples. For sampling and analysis to be acceptable to industry as part of BWC compliance monitoring, it will first be necessary to design, execute, and publish these validation experiments with academic, industrial, and government participation. This task may take years of intensive work, so the amount of effort involved should not be underestimated.

Investigation of alleged use. What if, a participant asked, a country claims to have

been attacked by biological weapons? Say it finds an unexploded munition on its territory and requests the international BWC inspectorate to determine what is inside. What methods would be used and how reliable would they be? Dr. Morse responded that the chosen methodology would depend on the nature of the munition. In the case of a ballistic missile warhead, for example, one would ask whether the type of warhead in question is likely to have a biological, chemical, or conventional high-explosive fill. An x-ray might help indicate what type of fill the warhead contains.

If the possibility of a biological fill is not ruled out, one would need to sample and identify the biological agent. The first step would be to contain the warhead so that no hazardous material is released into the environment. This could be a problem in the field, where there may be a shortage of containment facilities. If the warhead has already cracked open, then using appropriate precautions, a sample could be taken for analysis. Otherwise one would have to drill into the munition or remove the filling plug, as was done with chemical munitions during UNSCOM inspections in Iraq. Because x-raying the warhead would probably kill any living microorganisms, this technique would rule out the possibility of a bioassay. If no x-ray is taken, then all three analytical methods (bioassay, immunoassay, or DNA probes) could be used to identify the agent fill.

The following comment was received in writing from a pharmaceutical company representative after the workshop.

It is true that the more independent ("orthogonal") tests one performs, the less the likelihood of systematic error in identifying microorganisms. However, the real question is not whether a microorganism or toxin is present in a facility, but rather the *meaning* of its presence. In some parts of the United States, the concentration of anthrax bacilli in agricultural regions is about one to five spores per gram of soil, or 100 to 500 spores per 100 grams of soil. Because PCR is capable of detecting such trace quantities, it is almost certain that former stockyards—including the site of Eli Lilly's vaccine plant in Indiana—are "positive" for anthrax. Yet this is an *analytical* positive rather than a *diagnostic* one. An analytical positive has no meaning for BWC compliance monitoring in the context of a routine facility inspection.

As is well known in Bayesian statistics, the positive predictive value of a test is useful if, and only if, the false-positive rate is less than the pre-test probability. In practice, it is difficult to determine the actual false-positive rate of multiple orthogonal tests (caused chiefly by environmental contamination) and the pre-test suspicion (based on intelligence information). Yet it is precisely the pre-test suspicion that defines the meaning of a positive or negative test result. A positive finding of anthrax in an Iraqi facility is much more worrisome than one in a US facility because Iraq has already violated the BWC and thus has a much higher pre-inspection probability of a violation than the United States.

Until and unless the pre-test suspicion is available and the rate of false positives associated with environmental contamination is known (and in general, it is not), the use of sampling and analysis for compliance monitoring is meaningless—or worse, because it could lead to false accusations. Indeed, it can be shown that under some circumstances (i.e., low pre-test probability or high environmental contamination), a positive test result would provide less confidence in noncompliance than a negative result. This inherent ambiguity is why the US pharmaceutical industry objects to on-site sampling and analysis, except in those areas of a plant site where quality control has eliminated the possibility of contamination with environmentally abundant microorganisms,

such as the final processing lines of a commercial product.

Consider the following hypothetical scenario. A country previously known to have violated the BWC declares a facility that is ostensibly involved in the research and development of defenses against biological weapons, an activity not prohibited by the treaty. According to the declaration, this facility works with small quantities (ten grams or less) of approximately three dozen microorganisms and biologically derived toxins. Occasionally, the facility cultivates microorganisms to test vaccines in animals; to test the open-air survivability of microbial agents that it regards as potential threats to its population, livestock, or crops; or to test detectors and physical protection measures such as masks, suits, and decontamination fluids. When the facility is inspected, samples from inside fermentors, culture collections, surface wipes, and perhaps soil samples are positive for multiple microorganisms and toxins. Although PCR-based techniques make it possible to detect extremely small quantities of these materials, the meaning of this finding is completely subjective, because the intent, use, and actual quantities of the materials produced are unknown. Thus, sampling and analysis has provided no diagnostic information beyond what is already known from the facility declaration.

A BWC inspection team then visits a US pharmaceutical facility and employs the same battery of analytical techniques. PCR testing of laboratory-size fermentation vessels and surface swipes gives positive results for three toxins (diphtheria toxin, pseudomonas toxin, and botulinum toxin) and a few microorganisms (e.g., anthrax, plague). The pharmaceutical facility had declared all three toxins, but neither of the microorganisms. Although the facility claims that the two microbial agents are environmental contaminants, the test results are ambiguous. Thus, the results of sampling and analysis have left the inspectors in a quandry: they cannot find evidence of illicit activity at a suspected BW production facility, whereas a legitimate pharmaceutical plant appears to be in violation of the BWC.

In sum, on-site sampling and analysis during facility inspections either has no diagnostic value or may create confusion. There are only two circumstances in which sampling and analysis can be of diagnostic value: for investigating allegations of use of biological weapons and suspicious outbreaks of disease, such as the 1979 anthrax epidemic in the Soviet city of Sverdlovsk.[12] A diagnostic positive has meaning in this context, particularly if the source of the original fermentation culture can be identified and the strains compared with those found at the site of alleged use or obtained from victims of the disease outbreak. In such cases, little if any ambiguity would result from employing highly sensitive analytical tests to provide additional information.

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Lessons from the UNSCOM Experience With Sampling and Analysis

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3

The United Nations Special Commission on Iraq (UNSCOM) was established by the UN Security Council in the aftermath of the 1991 Persian Gulf War to uncover and destroy or render harmless Iraq's nuclear, chemical, and biological weapons of mass destruction and long-range missile delivery systems. In monitoring Iraq's biological capabilities and uncovering its proscribed biological weapons program, UNSCOM has employed sampling and analysis technologies. This experience has shown that while sampling and analysis can be an important adjunct to compliance monitoring, the sampling must be well defined, the time and place carefully chosen, and the analytical results put in perspective with information obtained from other sources.

Biological Monitoring in Iraq

In November 1993, the Iraqi government finally accepted the provisions of Security Council Resolution 715, under which its dual-capable industrial facilities would be subjected to long-term monitoring to make sure they would not be diverted to the production of banned weapons. To perform this mission, UNSCOM established the Ongoing Monitoring and Verification (OMV) program, which is carried out by resident monitoring teams based in Baghdad. Although OMV was initially implemented for the chemical, nuclear, and missile disciplines on October 1, 1994, biological OMV did not begin on an interim basis until December 1994 and was not fully implemented until April 1, 1995. The delay in implementing biological OMV was caused by Iraq's failure to declare all of its dual-capable facilities, requiring UNSCOM to carry out additional inspections to establish a monitoring baseline. Before January 1994, Iraq had formally declared only six dual-capable biological facilities, of which UNSCOM had inspected only a few. By September 1996, however, the Commission was monitoring 86 biological facilities throughout Iraq, including universities, breweries, food-processing plants, and production facilities for vaccines, antibiotics, biopesticides, and single-cell protein (an animal feed supplement).

Essentials of OMV

Biological OMV is based on facility declarations provided by Iraq and updated every six months. Under Security Council Resolution 715, Iraq must submit declarations for all sites

containing dual-use equipment or activities that are subject to monitoring. In addition to site identifiers such as address, director, and organizational chart, the declarations must include information on dual-capable equipment and microbial agents and toxins; imports, exports, and transfers of these items; collaboration with domestic and foreign organizations; and qualitative and quantitative estimates of activity at each site.

After analyzing the Iraqi declarations, UNSCOM performs no-notice inspections of declared sites, and sometimes undeclared ones as well. The resident biological team makes an *initial inspection* of new sites identified for monitoring to obtain a basic familiarity with each facility and to lay the groundwork for a more detailed *baseline inspection* by another team. (For smaller sites, the same team may perform the initial and baseline inspections.) The aim of the baseline inspection is to make sure the Iraqi declaration is accurate and that all relevant dual-use items have been declared. During on-site inspections, all areas of a site are subject to inspection and the facility is not allowed to control access. Based on the results of these inspections, new sites may be added to the OMV program.

UNSCOM obtains information from a variety of sources that significantly contribute to its monitoring capability, such as aerial photography by a U-2 surveillance aircraft loaned by the United States government. Information derived from Iraqi declarations, on-site inspections, aerial photography, and other sources goes into developing *site protocols*, the basic documents employed by resident OMV monitoring teams. These protocols are updated frequently by UNSCOM headquarters in New York and made available to the resident teams on computer diskette.

UNSCOM also operates the Baghdad Monitoring and Verification Center (BMVC) to provide logistical and administrative support and work space for resident expert teams in each of the relevant disciplines (biological, chemical, ballistic missile, nuclear, and aerial surveillance). At the BMVC, the resident biological monitoring team has office space and a room for sample processing. Each resident team consists of four to six scientists/technicians who serve a three-month tour. Because team members are typically provided by supporting governments at UNSCOM's request, the background of each is largely the prerogative of the supplying countries. Ideally, team members should have a master's degree or equivalent experience in microbiology or an allied science, and the team chief should have a medical or veterinary degree or a Ph.D. in microbiology. The commission tries to ensure that each team has an appropriate balance of academic, industrial, and diagnostic expertise.

Monitoring Activities

Biological OMV involves several complementary activities. In addition to the six-month declarations described above, the most important monitored sites must also submit monthly questionnaires, which are tailored for each type of site (university, pharmaceutical plant, etc.) and provide a more detailed, up-to-date, and focused picture of ongoing activities. Monthly questionnaires consist of five sections covering facilities, activities, personnel, connections to other establishments, and connections to other plants within the site.

The resident biological OMV team conducts random site inspections, whose frequency is determined by the perceived significance of the site. Monitored biological sites in Iraq have been classified in categories A through D, depending on priority. Category A sites are inspected weekly to monthly, B sites every one to three months, C sites every three to six months, and D sites at least once a year. Site inspections involve a thorough examination of all areas, discussions with personnel, verification of changes from a previous declaration or inspection, verification of monitoring parameters (from the site questionnaire), astute observation and, on occasion, sampling on demand of equipment, materials, and products. At seven dual-

capable biological facilities, remote video cameras also provide real-time surveillance and a permanent record.

In addition to resident-team inspections, UNSCOM headquarters in New York assembles nonresident teams of inspectors with appropriate expertise, who perform in-depth inspections of category A and B sites at least once a year. These teams are headed by a chief inspector with extensive prior experience in Iraq. As an important adjunct to these monitoring activities, UNSCOM tags, inventories, and tracks the movement of key items of dual-use biological production equipment and maintains a database of equipment at all sites.

The Role of Sampling

Under UN Security Council Resolutions 687, 707, and 715, UNSCOM has the right to collect biological samples at any time and any location. Such sampling is not conducted with preset specifications; instead, the resident OMV team is authorized to collect samples wherever and whenever it deems appropriate. The team is also encouraged to exploit the real-time monitoring feature of the video surveillance cameras to ask Iraqi plant workers to take samples from production vessels while being observed "live" from the BMVC. Sampling may also be performed by nonresident teams as the opportunity presents itself, or by teams specifically tasked to conduct sampling at particular sites. Final approval authority for all sampling rests with the UNSCOM Executive Chairman, who makes such decisions with the help of biological experts at New York headquarters.

With respect to UNSCOM's mission in Iraq, biological sampling has several roles. First, sampling may provide information on Iraq's proscribed BW program, including the past or continued involvement of a site in banned activities. Second, sampling may contribute to verification of Iraqi declarations on past biological-weapons activities. Third, sampling may be related to current activities, either to obtain evidence of renewed Iraqi efforts to reestablish proscribed BW activities or to deter them.

The type of samples collected and their origin may differ, depending on the intent of the sampling. For example, sampling to determine the site's involvement in the pre-Gulf War BW program has different parameters from sampling intended to deter a resurgent program. At a suspect past BW agent production site, samples might be collected from equipment (fermentors, centrifuges, driers); stored material (powders, frozen specimens); interior walls, vents, and drains; and external locations such as suspect field-trial locations or waste-disposal areas. In contrast, samples taken to deter a resurgent program would be collected from active development or production activities, such as small samples of liquid material from fermentors or flasks.

For investigating Iraq's past BW program, the key issue is not so much what and how to sample as *where* to sample. With current analytical technology, if a microorganism or its toxin product are present, they can be found and identified. Nevertheless, narrowing the target area where sampling should be performed remains the single most challenging problem. If the sampling domain is not limited, the costs of systematic sampling—both financial and political—would rapidly become prohibitive.

For example, sampling soil in areas where Iraq conducted BW field trials may yield valuable information about which agents were tested, either confirming what Iraq has declared or identifying additional agents that were not declared. If the location of these testing sites cannot be identified precisely, however, sampling is impractical and could well be counterproductive. Negative results might only mean that the wrong location was sampled, yet this false-negative could be exploited by Iraq as evidence that no undeclared BW agents were ever developed and tested. UNSCOM inspectors must therefore be prudent in exercising their right to sample.

The purpose of sampling under biological OMV is as much to deter illicit activity as

it is to verify Iraq's compliance with the UN Security Council resolutions. In this case, *where* to sample is less of a problem, because sampling is appropriate anywhere illicit activity is suspected or could occur in the future. *What* to sample is anything that is likely to provide evidence of Iraqi non-compliance with Security Council resolutions (such as specimens from fermenters or flasks, stored material, output of driers) or that will create uncertainty in the minds of Iraqi officials and thus help to deter illicit activities.

Environmental sampling includes real-time sampling of air, water, or soil to detect the ongoing production of BW agents. However, real-time sampling systems currently lack sufficient sensitivity to the spectrum of BW agents to be monitored, are limited in scope, and are likely to be prohibitively expensive. Current air-sampling technology, for example, is limited to a small set of agents and requires extended collection time, making it impractical for monitoring purposes. Water is unlikely to contain evidence of agents of interest, and the limitations of air sampling also apply. Nevertheless, sampling of soil and surfaces may be useful for obtaining evidence of past or present activity with prohibited BW agents.

Retrospective sampling to detect past development or production of BW agents applies to water, soil, and surfaces of interior walls, laboratory benches, and other objects. Samples may also be collected from the inside of biological production equipment such as a fermentor, drier, or centrifuge. If serious attempts have been made to clean the equipment and conceal previous activity, a more intrusive approach may be required that includes dismantling the equipment. The inspection team must make judicious decisions about when such intrusive sampling is appropriate, but it would probably be warranted at a site where illicit activity is believed to have occurred in the past. If one believes the illicit activity is ongoing, then other means of sampling should be considered, such as demanding aliquots (collected under UNSCOM supervision) from an active production line or taking samples of recent end-products or of stored materials.

Preparation of Samples

During the establishment of the Baghdad Monitoring and Verification Center, the biology section opted against analyzing samples on site because of the challenging requirements for biosafety and level of training entailed in operating such a laboratory. In particular, it was unlikely that personnel rotating through Baghdad on a three-month basis would be capable of performing complex biological assays, which must be standardized and controlled to yield reliable results. For these reasons, it was decided not to perform such analyses on site but to establish a room where OMV personnel could safely prepare and package biological samples for shipping to outside reference laboratories that were better staffed and equipped. Thus, the biological room at the BMVC is used only for sample storage, packaging, and preparation for shipping. (See box on facing page.) The biological room has an air-lock entry and operates under slight negative pressure to prevent dangerous microorganisms from escaping. It is also supplied with the essentials needed for sample processing, including a refrigerator, ultra-low temperature freezer, Class II biosafety cabinet, and an autoclave to sterilize wastes.

UNSCOM relies on supporting governments to provide laboratory support. Because sampling is neither conducted on a regular schedule nor with great frequency, standing arrangements with outside laboratories are not practical. Instead, generic nonbinding agreements have been arranged with supporting governments. To date, UNSCOM has made use of biological laboratories in three countries. Sample analysis by laboratories in two different supporting countries is preferred, particularly when the results may be controversial.

Sample-Handling Procedures

Airlines and customs officials are often uneasy about the shipment of biological samples, particularly when the sample in question is suspect material from Iraq. UNSCOM follows International Air Transport Association (IATA) regulations and has established a standard operating procedure (SOP) for the collection, packaging, and shipping of biological samples from Iraq. The sample is labeled as originating from Iraq and shipped internationally to the supporting reference laboratories.

For purposes of shipping and handling, biological samples collected in Iraq are designated as confirmation, diagnostic, or identification samples, or infectious substances.

- *Confirmation samples* are samples taken to verify declared activities at sites engaged in biological research or production.
- *Diagnostic specimens* are defined as any human or animal material shipped for purposes of diagnosis, including, but not limited to, excreta, secreta, blood and its components, or tissue and tissue fluids, but excluding live infected animals.
- *Identification samples* are of unknown materials collected from declared or undeclared sites in which UNSCOM suspects activities not consonant with the stated activity.
- *Infectious substances* are samples known or suspected to contain viable microorganisms that cause disease in humans or animals. Toxins known to be free of microorganisms are handled under rules for poisonous substances.

Samples are packaged in the BMVC biology room according to IATA regulations. All samples are placed in either sterile 2.0-milliliter Saf-T-Seal® microcentrifuge vials (for known or suspected infectious substances) or sterile 50-milliliter centrifuge tubes (for diagnostic or environmental samples). The tubes and vials are wiped with biocide, and the sample's unique serial number (assigned at the sampling site and recorded in the sampling data log book) is written on the side of the vial in indelible ink. Together with the signature of the inspector who drew the sample, this permanent reference number serves to identify the sample. The signature of an Iraqi representative is also desired to indicate that Iraq acknowledges the provenance of the sample.

The vial is then placed in a sterile sampling bag with a sheet of adsorbent paper, and the sampler signs and annotates the sample number across the seal at the top of the bag. This first sampling bag is wiped with biocide and placed in a second sampling bag. As with the first bag, the inspector taking the samples signs and annotates the second bag's seal with the sample's unique serial number. The double-bagged sample is then placed in a large zip-lock bag with a copy of the sample data sheet. Finally, the sampler stows the sample in a Saf-T-Pak® Infectious Substance Shipper prior to collecting the next sample.

To ensure chain-of-custody, packaged samples are placed in a Saf-T-Case that is then locked. One person designated by the chief inspector as responsible for the samples retains the keys to the lock(s). In addition, immediately prior to shipment from BMVC, a tamper-proof tag is placed on each Saf-T-Case. If control of the case is turned over to someone else before the tag is attached, then the receiving person signs for the samples and the collection team retains the receipt form as a legal document. Accompanying each set of samples is the data sheet, which provides information about their origin and how the samples were collected. This data sheet is returned to UNSCOM Headquarters in New York at the conclusion of the mission. When this SOP has been followed, UNSCOM has encountered no problems in shipping samples from Iraq.

Obtaining entry certificates for the samples is the responsibility of the supporting laboratories. In addition, arrangements for sample processing are made by UNSCOM in New York prior to shipping the sample. Sample assay methodologies are chosen by the supporting laboratories in consultation with UNSCOM staff to meet the goals of the particular sampling mission. The requirement for baseline and control samples is a function of the nature of sample collected and the test to be performed. Baseline data are less important for on-site sampling procedures than if UNSCOM were taking environmental samples of air or water. Obtaining control samples when so requested by supporting laboratories has not been a problem.

Results of Sampling in Iraq

Iraq has acknowledged having pursued research and development for BW purposes on several bacterial pathogens (*Bacillus anthracis*, *Clostridium botulinum*, *Clostridium perfringens*, *Fusarium sporotrichioides*, *Aspergillus sp.*) as well as three viral agents (camel pox, human rotavirus, hemorrhagic conjunctivitis virus). The Iraqis have also admitted to weaponizing anthrax spores, *Clostridium perfringens* spores, botulinum toxin, and aflatoxin, a fungal toxin that is a potent carcinogen and a possible incapacitant at high doses. To date, however, the Iraqis have denied studying other microbial or toxin agents for BW purposes. Sampling in Iraq could be complicated by the fact that the agents mentioned above, and others, are endemic to Iraq. Thus, sampling for evidence of BW activities must consider the environment in which the sample was taken and its correlation to a suspect site or activity.

Sampling has been and will be performed in Iraq when, in the opinion of UNSCOM, the circumstances warrant. In a few cases, onsite sampling at dual-capable biological facilities has provided strong circumstantial evidence of illicit activities. For example, before UNSCOM obtained conclusive documentary evidence that the Al Hakam Factory had been involved in Iraq's past BW program, sampling revealed some striking anomalies. Iraqi officials claimed that Al Hakam was engaged in the legitimate production of *Bacillus thuringiensis*, a bacterium that produces protein crystals with insecticidal activity. Yet sampling of the spray dryers at Al Hakam in December 1994 and June 1995 revealed that although the final product was indeed *Bacillus thuringiensis*, the bacterial cells did not contain pesticide crystals. This finding suggested that the declared production was a facade and that its real purpose may have been as a training exercise for the cultivation of anthrax bacteria, which grow under similar culture conditions. Moreover, the finished dry form of *Bacillus thuringiensis* had a particle size of approximately 10 microns, too fine for a biopesticide—given that the powder would not settle out of the air on crops—but suitable for the creation of a respirable biological aerosol, a requirement for the large-scale dissemination of BW agents.

In 1994, the equipment and surfaces of Al Hakam were extensively sampled and tested for live BW agents, with negative results. In addition, environmental samples taken from soil, the interior of buildings, and sewers at Al Hakam were negative when cultured for suspect microorganisms. In 1996, however, equipment and surfaces at two other sites were sampled and tested using the polymerase chain reaction (PCR) technique, with positive results. In addition, seed cultures that Iraq claimed had been inactivated before being buried in the desert in 1991 were analyzed by bioassay, immunoassay, and PCR. Although the bioassays were negative, immunoassay and PCR were positive for the declared agents. Prototype aerosol generators tested by immunoassay and PCR were also positive for an agent declared by Iraq. These results were useful, but they did not reveal "smoking-gun" evidence of undeclared activities that could not plausibly be explained away.

The Political Dimension

Thus far, the Iraqi government has cooperated fully with UNSCOM's sampling activities, and site personnel have not raised other than mild objections. Although UNSCOM has not attempted to exploit the full scope of its sampling authority, this may change when the economic sanctions on Iraq are lifted and the capabilities of its dual-capable biological production facilities improve. If and when sampling becomes more intrusive, Iraqi objections are likely to increase. At the same time, the Iraqi authorities have sought to use the results of sampling and analysis to serve their political ends, usually as evidence to claim that they are complying fully with their obligations under the cease-fire agreement.

The political aspects of sampling must not be overlooked. Sampling may entail political

costs, particularly when the target country seeks to undermine the credibility of the inspection. Before joining the UNSCOM staff, I considered myself a nonpolitical person. Yet every activity the commission contemplates must be scrutinized for its potential political implications. Before a sample is collected and analyzed, we must be sure that either a positive or negative result will have some meaning. If a sample is found to be positive for any of several biological agents, so what? Iraq may have declared those agents, and the analytical results cannot tell us the amounts produced, which is the real issue relevant to compliance. If the results are negative, so what? The target area is sufficiently ill-defined that perhaps we are not sampling in the exact location or collecting the right type of sample. In some cases, UNSCOM has chosen not to sample because the meaning of either a positive or negative result was unclear. In other cases, UNSCOM inspectors have gone ahead with sampling even when the significance of the results was questionable, simply to reaffirm the commission's right to sample and to help deter future Iraqi violations.

When considering sampling, however, UNSCOM must decide whether the political cost may be prohibitive. Iraq has been particularly skillful at using disinformation techniques to twist seemingly trivial events to its political advantage. In 1994, for example, UNSCOM conducted a biological audit of the Al Hakam Factory in which samples were collected from fermentors and other equipment, at various locations within the production buildings, and from sewers and septic systems. The sampling took place at a time when we were trying to persuade the Iraqis to acknowledge their past BW production activities, including the role of the Al Hakam Factory. Yet Iraq tried to convince some members of the UN Security Council that there was nothing to UNSCOM's allegations by claiming that we had sampled "all over Hakam including the toilets" and found no evidence of misdeeds. Considerable effort was needed to counter this perception. Later the same month, an inspection at Al Hakam obtained samples providing the first indication that Iraq was producing a small particle-size *Bacillus thuringiensis* product lacking pesticidal activity.

In conclusion, UNSCOM views sampling and analysis not as an end in itself, but as an important adjunct to the investigation of Iraq's proscribed biological program under Security Council Resolution 687 and to monitoring its capability to re-establish an illicit program under Security Council Resolution 715. If performed under the appropriate conditions, sampling can help verify Iraq's declarations and ensure that its dual-capable biological research, production, and test facilities are employed only for legitimate purposes.

Discussion

Iraqi complacency. One participant noted that although Iraq was skilled at hiding actual biological munitions, none of which has ever been found, UNSCOM was still able to find strong evidence of suspicious activities—most notably, the production of a biopesticidal bacterium lacking pesticidal activity and having a particle size more appropriate for biological warfare than for agricultural use. Did Iraq not realize how effective the sampling effort was? Dr. Spertzel responded that one of the reasons for UNSCOM's success in the biological realm was Iraqi complacency. From 1990 to 1994, only three dedicated biological inspections took place in Iraq. As a result, Baghdad developed a feeling of confidence and perhaps did not believe that biological sampling would take place.

Blood samples. One participant asked whether UNSCOM had taken any blood samples from Iraqi plant workers to test them for antibodies to known BW agents. Dr. Spertzel responded that no blood samples had been taken because of the legal and human-rights barriers, including likely

resistance from the UN Security Council. If UNSCOM could identify stored samples of plant workers' blood or sera, these might be tested. But the Iraqi government, which claims that plant personnel were not immunized, would probably deny that blood samples existed, whether they did or not. Dr. Spertzel added that the most likely sites where blood samples might be stored have already been inspected.

Time needed for clean-up. A participant asked roughly how long it would take Iraq to remove all traces of BW agents from its biological production equipment. Dr. Spertzel replied that Iraq cleaned its equipment with a mixture of formaldehyde and potassium permanganate, which did a very thorough job, but that the equipment first had to be dismantled. Most of Iraq's fermentors had plug-in segments that could be rapidly connected and disconnected for greater flexibility. How long it took to dismantle and sterilize the equipment is uncertain, Dr. Spertzel said, but a reasonable guess is a day or two.

Utility of sampling. One participant noted that since Iraq has declared that it has produced certain BW agents for legitimate purposes (such as anthrax for vaccine production), finding traces of these agents would not provide any additional information, while not finding them could simply mean that samples were collected in the wrong place. The questioner asked why UNSCOM bothered to sample if the results could not be interpreted unambiguously, unless the purpose was merely to affirm the commission's right to sample. Dr. Spertzel replied that if there were grounds to suspect that Iraq was producing agents other than what it had declared, it would be worthwhile to sample for the undeclared agents and risk a negative finding. Indeed, rumors and unverified intelligence suggest that at least one site, Iraq produced a BW agent that it has not yet acknowledged, so UNSCOM intends to take samples there. Nevertheless, the commission cannot afford to make repeated allegations about illicit BW agent production that are not corroborated by sampling and analysis. After several negative results, the commission would lose all credibility with the UN Security Council.

Purpose of sampling. A participant asked whether UNSCOM took samples to confirm that the microbial agents declared by Iraq were present, or rather to identify undeclared BW agents that might be present in the samples. Dr. Spertzel said that both approaches were used. Although UNSCOM is obligated to verify Iraq's declarations, the samples were analyzed for a variety of BW agents, both declared and undeclared. The questioner followed up by asking about the costs and technical difficulties of analyzing a sample for its contents without exclusionary criteria, rather than simply eliminating possibilities from a list of known agents. Dr. Spertzel replied that UNSCOM has not tested samples for all possible microbial pathogens but instead has looked for the standard list of BW agents. In principle, however, it would be preferable to identify exactly what a sample contains and only then draw conclusions.

A participant noted that although identifying unknown microorganisms in a sample is a nontrivial task, environmental microbiologists have made considerable progress in identifying the microbial content of soil samples. The universe of putative biological warfare agents is relatively small because countries seeking a BW capability would only wish to develop the fraction of microbial pathogens that can be produced in large quantities and weaponized. Thus, one could probably afford to do a fairly exhaustive screening of samples for likely BW agents.

Sampling procedures. Dr. Spertzel was asked about UNSCOM's standard operating procedures (SOPs) for sampling and analysis. Although the SOP for packaging and shipping is well established, what about the sampling protocol? Are inspec-

tors trained in methods for taking specific types of samples, and has UNSCOM used the same sampling procedures at Al Hakam and other sites? Dr. Spertzel replied that UNSCOM inspectors are familiar with basic microbiology and are sufficiently trained to handle basic sampling procedures, which vary depending on the type of sample they seek to collect. The SOPs apply less to the act of collecting the sample than to their handling, documentation, and the conditions under which they are stored. In particular, it is essential to establish a forensic paper trail (chain of custody) to settle any future disputes about the authenticity of the samples and whether they have been tampered with.

Industry Views on Sampling and Analysis

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4

The US pharmaceutical industry intends to participate actively in the development of a compliance protocol for the Biological Weapons Convention (BWC). Although international inspections of industrial facilities are unlikely to yield conclusive evidence that biological or toxin agents have been produced for illicit purposes, such inspections could serve as a deterrent by increasing the costs and political risk of clandestine production. At the same time, any BWC compliance regime must safeguard commercial proprietary information (CPI), which is the lifeblood of our industry.

The central mission of research-based pharmaceutical companies is to discover, develop, and manufacture proprietary medications. These firms have strong incentives to return value to their shareholders by producing high-quality drugs for the consumer. No Western company, from a small startup to a pharmaceutical giant, has any economic interest in participating in the development or production of biological weapons. To contend otherwise would be fatuous, and frivolous or malicious allegations directed against the pharmaceutical industry for political or commercial reasons could cause us great harm.

The following analysis has been drawn from my own views and from discussions with visitors to my workplace, Eli Lilly & Company, over the past few years. This analysis was reviewed and strengthened by comments from representatives of member companies of the Pharmaceutical Research and Manufacturers of America (PhRMA), the leading trade association of the research-based, ethical pharmaceutical industry in the United States. PhRMA represents more than 100 pharmaceutical companies that discover, develop, and manufacture prescription drugs and biologics such as human insulin and vaccines. Although this paper is not an official PhRMA document, it reflects the views of the association's Subcommittee on the BWC.

The Value of CPI

Pharmaceutical and biotechnology companies seek to safeguard proprietary information on manufacturing processes (including culture media and procedures), inventories, equipment and volumes, production capacity, raw material supplies, distribution and marketing plans, product stability, and registration plans.

Companies intending to bring a new product to market also seek to keep confidential the results of ongoing clinical trials.

On-site sampling by an inspection team during the production of a biotechnological product could compromise the following types of CPI: (1) the species and strain of the production microorganism; (2) the identity of the plasmid, or ring of DNA incorporating the recombinant gene for the product; (3) the relevant coding sequences in the plasmid DNA; (4) the precursor product (e.g., proinsulin); (5) specific steps in the manufacturing process; and (6) unannounced new products. The compromise of some or all of this information could seriously erode a company's competitive edge, resulting in the loss of millions of dollars in sales. Indeed, a single genetically engineered bacterial culture may be worth as much as $1 billion.

In some biotechnology markets, competition is extremely aggressive and companies exploit every advantage to promote the sale of their products over those of competing firms. These individual markets may range in value from $100 million to more than $2 billion for a single product. Thus, the potential loss or gain of a few percentage points in market share is sufficient to motivate a large biotech company to protect its CPI at all costs. From another angle, it costs a large pharmaceutical house between $350 million and $500 million to bring a new pharmaceutical product to market. Any loss of sales against that level of investment would be a serious financial blow, one that many companies could not weather successfully. With such huge investments at stake, companies are determined to protect their CPI.

Because of these compelling economic interests, sampling in any form is anathema to the pharmaceutical industry. I think most of us feel that no matter how innocuous an on-site inspection and sampling regime may appear, we are sure to lose significant CPI in the course of a challenge inspection. Thus, the economic value of this information must be placed in the balance. Although US companies would prefer by far that there be no sampling whatsoever, we recognize this stance is unrealistic in a world in which some countries do develop and produce biological weapons in violation of international norms.

Managed Access Procedures

While it is clear that no pharmaceutical company would welcome an international inspection team with open arms, individual plants differ in their level of comfort with respect to on-site sampling. Similar industry concerns with respect to on-site inspections of chemical plants under the Chemical Weapons Convention (CWC) led to the development of the concept of "managed access," in which the inspection team and the inspected facility negotiate the amount of access to be provided to sensitive areas of the site, so as to resolve concerns about treaty compliance without jeopardizing CPI.

Managed access involves a negotiation process between the inspection team and the inspected facility (in conjunction with officials of the state party) to determine what information will be made available, who may be interviewed, which buildings may be entered, what equipment may be examined, what computer and written records may be audited or copied, where photographs may be taken, and whether other information may be accessed by the inspection team. Such procedures can be applied during any type of on-site inspection, including a short-notice or challenge visit.

In some cases, the inspected facility may decide to deny the inspectors access to a certain area for a number of reasons, including safety hazards, operational considerations (such as the need to maintain sterile conditions), or protection of CPI. If access is denied or restricted, the onus is on the inspected party to provide alternative types of information that will resolve the inspectors' compliance concerns. Failure to address these concerns may lead the inspectors to conclude that the inspected facility is trying to hide il-

licit activities, although the presumption of innocence must be rigorously applied.

Sampling and analysis will almost certainly be subject to managed access. Serious negotiation will be needed to determine which requested samples can be provided and which cannot, and in the latter case, what alternative types of evidence are acceptable. PhRMA believes that if managed access is implemented in good faith, it can meet the needs of both industry and the inspection team. While neither side may be fully confident that its concerns are being met, the two should be able to reach a compromise that allows the inspection process to move forward.

Where will the inspection team want to take samples? In principle, sampling in a pilot plant or a manufacturing area could occur wherever there is a volume of liquid or a solid surface. Such locations include, but are not limited to (1) the contents of the production vessel; (2) the contents of the seed vessel; (3) the condensate collection vessel; (4) the outsides of these vessels; (5) piping and flanges; (6) ball, diaphragm, and control valves; (7) air filters; (8) sample lines; (9) the culture-raising laboratory, including culture collections, incubators, shaken cultures, transfer vessels, refrigerators, and materials to be autoclaved; and (10) benches, sinks, floors, and walls throughout the facility, including laboratories and fermentation halls.

Sampling and analysis might be conducted in three different ways: on-site analysis by the inspection team or designates; off-site analysis by the inspection team or designates; and on-site analysis requested by the inspection team and carried out by site employees. The optimal procedure from industry's standpoint would be scenario-dependent. Suppose a challenge inspection leads to requests for sampling at a Western pharmaceutical company. Could we permit samples to be removed from the premises for analysis? I would like to say "no" across the board, but realistically there are some samples that when analyzed would not give away CPI and that we would have no concern about leaving the premises. A trivial example is a common bulk ingredient such as dextrose. Similarly, the bulk product emerging from the end of the production line is not commercially sensitive and could be provided for off-site analysis.

As a general rule, however, the inspected plant must have the right to indicate which samples are suitable for removal from the premises and which are not. Because it is unlikely that the inspection team would be prepared to perform an off-site analysis, a reference laboratory would be designated to perform this task. To be acceptable, this laboratory would have to be approved by both the inspection team and the inspected party. The inspected company may also wish to send representatives to the designated laboratory to observe the analysis.

For materials of a more proprietary nature, such as a novel raw ingredient, the inspected company would probably not resist the inspection team's request to take samples. Such materials could be analyzed on site to demonstrate that they do not contain suspect BW agents. At the same time, the company would not want the sample to leave the premises so that its precise composition could be analyzed. (Although knowing the identity a novel raw material is not the same as a detailed understanding of its use in the production process, such information might be obtained from discussions with scientists, technicians, and operating staff, some of whom may have no idea of the value of the information they possess.)

Finally, some samples contain highly sensitive CPI, such as broth containing the production microorganism or a plasmid preparation. In such cases, suitable assays would have to be carried out by company employees in the presence of the inspection team, who would not be allowed to touch the sample in any way. Testing to demonstrate that the sample is not of a proscribed nature should be sufficient to satisfy the inspectors, particularly if the tests have been agreed on

in advance by both parties and have the specificity needed to ensure unambiguous results.

The suggestion by the Federation of American Scientists (FAS) Working Group on Biological Weapons Verification that proprietary samples be taken off site when "an essential test to address a specific concern cannot be performed on site, or … confirmation of on-site analytical results is considered necessary" is unacceptable to industry because it does not ensure protection of CPI. Furthermore, the FAS Working Group's assertion that the removal of killed microorganisms for off-site analysis would pose no risk to CPI is seriously flawed. In fact, pieces of DNA containing highly proprietary gene sequences could be recovered from nonviable cells that are no longer capable of division.

If the inspected facility judges the risk of lost CPI as negligible, it could release samples to the inspection team for off-site analysis through the managed-access negotiation. Industry would prefer, however, that technicians from the inspected facility perform all sample analyses on site, employing procedures specified or agreed to by the inspection team.

Should it become necessary for a member of the inspection team to conduct a particular test, the inspected party should have the right to inspect the assay procedure thoroughly. In addition, test validation reports must be available to the inspected party. At a minimum, the assay's limits of detection and quantitation must have been determined and found acceptable prior to the inspection, including rates of false positives and false negatives. Finally, the robustness of the assay must have been established with respect to possible background influences. These three criteria of suitability—sensitivity, specificity, and robustness—could disqualify many candidate assays.

Prevention of Covert Sampling

Beyond the issue of authorized sampling and analysis during on-site inspections, the pharmaceutical industry is concerned that members of an inspection team might engage in covert sampling activities that could result in the loss of CPI. This perceived threat can be divided into two scenarios: (1) the inspection team may conspire collectively to obtain covert samples by whatever means at their disposal, or (2) an individual member of the team may seek to obtain a covert sample for purposes of industrial espionage.

Because the production microorganism (containing the recombinant genes) is of primary economic value to industry, protecting it against covert sampling is critical. It is possible but unlikely that a clandestine sample of laboratory air could contain the production microorganism. In this case, there is little the inspected facility could do to prevent covert sampling because miniature air-sampling systems are surely available. To prevent covert sampling by most other means, however, the inspected facility should provide an escort for *each member* of the inspection team, including interpreters and administrative assistants. Besides helping to guide the inspectors around the plant and answering questions, the escorts' function would be to keep the inspectors from touching materials, production equipment, and other surfaces. Materials being prepared in a hood or stored in a refrigerator would be completely off limits.

Another effective means of preventing covert sampling would be to require the inspectors to remove their street clothes and don facility-supplied coveralls, booties, a head covering, and a surgical mask, which would be destroyed after use. The inspectors would also be required to shower after the inspection. Finally, the inspected facility should have the right to demand the removal of any inspector caught or suspected of taking unauthorized samples.

Results of Trial Inspections

Several trial inspections of pharmaceutical facilities have been conducted over the past few years.[1] In each case, despite a consider-

able amount of preparation time, serious breaches of confidentiality occurred because of deliberate efforts by the mock inspection team and naiveté on the part of the staff of the inspected facility. Not only was it difficult to limit the access of inspectors to defined areas, but they managed to discuss a range of sensitive topics with junior plant workers, who were all too eager to engage in technical discussions and revealed more than was necessary. By piecing together bits of information gleaned from such conversations, the inspection teams were able to deduce correctly a good deal of proprietary information about the inspected facility. The inspectors were also free to take photos, examine notebooks, and collect samples. While none of these activities should be proscribed outright, they should be part of the managed-access negotiation between the inspectors and a team of trained company escorts who have been fully briefed about the facility's CPI concerns.

Industry Confidence in Sampling and Analysis Techniques

All analytical techniques have strengths and weaknesses. The enzyme-linked immunosorbent assay (ELISA), for example, may indicate the presence of specific antigens—distinctive portions of proteins on the surface of a microorganism. Some of these antigens, however, may be present both on a pathogenic BW agent and on a nonpathogenic microorganism that has been genetically engineered to produce a natural compound. As a result, an ELISA test could yield a false positive that might unfairly implicate a legitimate pharmaceutical company in a violation of the BWC.

Similarly, the detection of specific gene sequences by "genetic fingerprinting" is not the same as the unambiguous identification of a BW agent. Because closely related microbial species often have large stretches of homologous DNA, false positives are bound to occur. Finally, some pathogens such as anthrax spores are ubiquitous in soil at low concentrations and could easily be introduced inadvertently into nonsterile areas of a pharmaceutical plant on the bottom of workers' shoes. For these reasons, various assays may have unpredictable levels of false-positive and false-negative outcomes.

The FAS Working Group contends that Fourier-transform infrared (FTIR) spectroscopy will eventually be capable of identifying microbial pathogens, if not as single cells than as colonies growing on agar plates. While I would not rule this out, it will take several years before the technology has progressed to the point that it has such a capability. Finally, although the polymerase chain reaction (PCR) is one of the most powerful techniques available today for amplifying small samples of DNA to detectable levels, it cannot identify a BW agent unambiguously. Even multiple DNA fragments, successfully amplified from the same source, will not provide total confidence. The reason is that the amplification of common base sequences does not enable one to identify the presence of a BW agent with certainty. Moreover, nonprotein toxins, which are produced by microorganisms through a series of biosynthetic pathways, may not be detectable with DNA probe/PCR techniques.

Many analytical techniques occasionally give false positives, for the following reasons: (1) the test may be at the limit of sensitivity for the analytical method; (2) the test may identify common genetic or antigenic elements present in both a pathogen and a legitimate production microorganism; (3) the test may detect the presence of a pathogen that is ubiquitous in trace amounts in the environment; or (4) the samples may be contaminated—either accidentally or deliberately—with standards used to validate the assay. Although there are other potential sources of false-positive results, these four mechanisms are the major ones.

Careful use of controls and intelligent interpretation of the data can increase confidence in the results of sampling and analysis. In particular, it is strongly advisable to confirm the presence of suspected agents by

using "orthogonal" assays, meaning two analytical methods based on different scientific principles. If two orthogonal assays yield the same result, one can begin to have confidence in the accuracy of the findings. Even so, one must ensure that the two techniques really come at the problem from different angles and are not mirror-images of each other. For example, if a single gene is identified with PCR and the protein it codes for is identified with an immunoassay, one is no closer to the unambiguous identification of a BW agent. A final note is that while the analytical techniques discussed here may appear very powerful, they are of questionable value as long as they remain unvalidated for this specific purpose. Validation must be a prerequisite for employing assays for any purpose, including BWC compliance monitoring.

Other Industry Concerns and How They Might Be Addressed

Although loss of CPI is industry's main worry about sampling and analysis, we have other concerns as well. Adverse publicity about a challenge inspection at a US pharmaceutical plant could create a public misperception that the company is engaged in illicit activities, a perception that could be exacerbated if a test for a suspected BW agent gives a false-positive result. Because all major pharmaceutical houses trade on their good name as developers and manufacturers of high-quality medications, the release of erroneous information implying serious wrong-doing could cause irreparable harm to the company's relationship with its shareholders and its reputation with the general public. Such negative publicity could cause the company's stock price to fall and drive consumers to adopt competing products. Even a malicious or frivolous request for a challenge inspection at a company plant—perhaps in retaliation for an earlier US challenge request—could impose a serious economic cost on the targeted firm.

Industry would receive some protection against unwarranted damage to its reputation if the Ad Hoc Group were to adopt a "green-light" approach to challenge inspections. Under this approach, the state party requesting a challenge inspection would have to provide compelling evidence of a violation before a majority of the "executive council" (a representative body of BWC states parties) could vote to allow the inspection to proceed. If challenge inspections could be requested only on strong suspicion of a violation, then US industry would have no objection, because such activity by a Western pharmaceutical or biotechnology company is unthinkable.

Conclusions

PhRMA member companies are legitimately concerned about on-site inspections and associated sampling and analysis activities. We support a mechanism for challenge inspection as one element of a compliance-monitoring regime for the BWC and endorse the use of managed access to handle requests for sampling and analysis in the same manner as other requests for proprietary information. We do not believe, however, that available analytical technologies can provide adequate confidence with respect to the presence or absence of BW agents to justify the risk that sampling poses to CPI at the inspected sites. Accordingly, we believe there is a need to develop validated analytical techniques, together with procedures for their use, that are acceptable both to the international inspectorate and to the pharmaceutical industry.

Discussion

Managed access. Many participants expressed concern about the concept of "managed access," in which the inspection parameters would be negotiated for each individual facility. A questioner asked how the inspection could proceed in a timely fashion if the managed-access negotiation became deadlocked. Dr. Muth replied that most facilities in the West would strive to work out a compromise promptly. In a

challenge inspection, the inspectors would presumably have evidence of a violation that they wished to investigate. To this end, they would seek access to particular areas of the plant and might ask to take samples. The facility managers might respond, "Items one and two on your list are acceptable, but you may not take a sample at the proposed location. As an alternative, I'll show you some regulatory documents describing our production." In this way, the two sides would hammer out a mutually acceptable plan.

A participant noted, however, that the regime includes many non-Western countries, including known BW proliferant states, for which this approach won't work. What recourse would the inspectors have if a satisfactory outcome to the negotiation could not be reached? Even worse, couldn't the managed-access negotiations enable a proliferator to stall for time and destroy all evidence of illicit BW agent production before the inspection began? Dr. Muth conceded that this delay could represent a drawback of managed access from the verification standpoint. He added, however, that this was not his problem, and that industry's proprietary interests must be protected.

Preparing for inspections. Dr. Muth stressed that pharmaceutical companies will need to learn how to manage inspections at their facilities so as to protect CPI. This task will include establishing a team of company escorts who are trained in managed-access techniques. He suggested that neither senior managers nor scientists would provide very good foils for the inspection team: whereas managers tend to be unduly secretive with outsiders, scientists and technicians are generally too open. Unthinking statements by junior technical staff may reveal sensitive proprietary information, such as how often fermentors are turned around or how many are used for a particular process.

Minimizing loss of CPI. The following suggestions were made for ways to reduce the risk that CPI could be compromised during an inspection:

- Inspectors could use throw-away microchip sensors containing DNA probes that are capable of detecting only microbial and toxin agents relevant to BWC compliance, and could be left behind at the facility after use like a disposable pregnancy test. Indeed, a representative of the Defense Advanced Research Projects Agency (DARPA) noted that her agency is funding the development of such devices.
- The inspection regime might be structured so that inspectors who visit a given facility would not know in advance which site they would be visiting, making it more difficult for them to plan for industrial espionage.
- The inspection team should not include nationals from the country being inspected, so as to reduce the risk that an inspector from a competing company would be allowed on site.
- Using the precedent of the Chemical Weapons Convention, the BWC compliance protocol should include a provision giving each state party the right to veto in advance any individual inspectors from participating in inspections on its territory.
- One participant noted the advantage of a professional inspectorate over a group of ad hoc inspectors because professionals would have fewer temptations to engage in industrial espionage. At the same time, a professional inspectorate might imply the need for nonchallenge inspections because it would be hard to justify the cost of a standing inspectorate that does not have regular inspection duties. A professional inspectorate would also need to conduct frequent inspections to keep its members busy and sharp. Dr. Muth said that he understood this argument but did not want inspectors trained at industry's expense.
- A private insurance fund could be established to indemnify industry for the value of any lost CPI.

Challenge vs nonchallenge inspections. Dr. Muth said while US industry would prefer no inspections of its facilities, it is prepared to accept a compliance regime in which only challenge inspections are carried out under a "green-light" procedure, which would require explicit approval of each request by the supermajority vote of an executive council. A nonindustry participant noted that because a challenge inspection would involve a serious allegation of a treaty violation, it would be in a company's best interest to cooperate fully with the inspectors to prove the allegation false and get them away from the site as quickly as possible.

Other participants stressed the utility of "nonchallenge" inspections of declared facilities, which might be carried out on a semirandom basis. Challenge and nonchallenge inspections would represent different regimes. A nonchallenge inspection would involve a routine check of the facility declaration and would be low-key and nonconfrontational, with little if any opportunity for sampling. In contrast, a challenge inspection would involve the pursuit of a suspected violation and hence would be conducted in a more intrusive manner. If noncompliance concerns arose during a nonchallenge visit, the facility managers could tell the inspection team, "You are exceeding the realm of a nonchallenge inspection, because CPI is now at risk. You must request a challenge inspection to see that area." The challenge request would then need to be backed up with compelling evidence of noncompliance.

Some nonindustry participants argued that nonchallenge visits would provide a powerful means of reducing suspicion and increasing confidence in BWC compliance. Moreover, in the absence of a nonchallenge regime, even relatively minor infractions could lead to a request for a challenge inspection, which would be more intrusive and open-ended and thus more problematic for industry. Arguably, industry could better protect its interests during a nonchallenge inspection, which would permit more control by the inspected party. Dr. Muth replied that he could not conceive of requests for challenge inspections at Western pharmaceutical plants because there would be no compelling evidence of a BWC violation.

Validation of assays. Dr. Muth pointed out that for a facility to confirm what it says it is doing, it can employ standard assays that it uses every day and that are available on site. The facility has validated these assays and understands their parameters for accuracy. In an accusatory framework, however, the inspection team would have to bring its own assays (e.g., for anthrax or other suspected BW agents). These assays would then have to be recalibrated and validated to demonstrate their accuracy at the facility in question.

Indeed, one of industry's main concerns about sampling and analysis is the need for validation of assays. Part of the license-application process in the United States involves proving to the federal Food and Drug Administration (FDA) that every assay performed to monitor the production process has been validated. Dr. Muth noted that it generally takes a few months to validate an assay under the best circumstances, and that revalidation may be required if the production process moves from one plant site to another. Another industry representative added that a pharmaceutical plant must demonstrate to the FDA that its assays have been fully validated or it will not be allowed to continue production. Thus, US companies are concerned that if BWC inspectors are allowed to run assays that do not meet the same level of stringency, this could violate FDA regulations and stop the flow of product for as long as a few months. It was agreed that, to address these issues, representatives from FDA and regulatory agencies in other countries should participate in the negotiation of the BWC compliance protocol.

Goals of inspection regime. An industry representative pointed out that the United States has the largest and technologically most advanced biopharmaceutical industry in the world, at the cutting edge of research and development. As Dr. Spertzel's presentation made clear, the protracted UNSCOM biological inspection regime in Iraq was unable to find a "smoking gun." Thus, the policy consideration is whether the cost of on-site measures outweighs the benefits. Policymakers must balance the probability of finding conclusive evidence of noncompliance against the likely economic costs to a leading US industry.

A nonindustry participant responded that seeking to find a smoking gun is an unrealistic goal. Instead, the BWC compliance regime should aim to enhance transparency, build confidence in compliance, and deter violations. If a protocol is developed that allows proliferators to stall a challenge inspection indefinitely, that would be unacceptable. Some mechanism must be created to ensure access to suspect sites, while protecting the commercial interests of Western industry.

An industry representative argued for allowing facility staff to conduct all sampling and analysis activities in the presence of the inspection team. However, a participant with UNSCOM experience noted that no inspector who had worked in Iraq would trust any test done by an Iraqi company on its own. He stressed that the provisions of the BWC compliance protocol will apply not just to Western countries but to all states parties, including some known or suspected BW proliferators.

Risk of industrial espionage. One participant asked Dr. Muth to compare the risk of an inspector stealing a sample and selling it to the competition, compared with a disgruntled employee doing so. Dr. Muth replied that insider thefts of CPI happen very rarely because of a sense of "fraternity" within the industry, which causes even strong competitors to band together against industrial espionage. Yet another industry representative recounted the story of an Italian executive who toured a competitor's plant, dipped his handkerchief in some broth containing a proprietary microorganism, and used it to market the same product a few years later. The moral of the story is that industrial espionage does occur, and that US industry cannot afford to take any chances.

One participant noted that because the inspectors would not know the identity of a challenged facility far in advance, it would be hard to plan for this kind of industrial espionage. Dr. Muth replied that some kind of collusion could occur at the planning stage.

Views of other countries. One participant observed that Australia, Canada, and several European countries favor nonchallenge inspections and claim they have the support of their national industries, yet the US pharmaceutical industry takes a very different position. Dr. Muth replied that he was not convinced that industry associations in other Western countries really understand what is at stake. He added that the member companies of PhRMA are afraid of going into a situation in which they may not be in control.

A participant responded that he had heard two different concerns expressed by the industry representatives, namely, "loss of control by having inspectors on site" and "the taking of samples." He argued that there is a tradeoff between these two concerns. In trying to avoid sampling, an inspected facility may have to accept a greater number of inspectors on site. With fewer inspectors, there might be a higher probability of sampling. Dr. Muth agreed that in the real world there will be inspections and sampling, but they should be managed to ensure the minimum risk to CPI.

Role of industry in negotiations. Participants discussed the appropriate level and timing of cooperation between govern-

ment and industry in developing detailed proposals for the BWC compliance protocol. Some participants thought early involvement by industry representatives in the process would be productive, because the more government and industry are aware of each other's concerns at an early stage, the better the final product can be. Close government-industry cooperation can also make it easier to gain industry support for the BWC compliance protocol during the Senate ratification process. Another group of participants argued, however, that it would be premature to engage industry before the US government has worked out its own positions in the interagency process. Both sides would end up expending time and energy to address issues that, at the end of the day, might not even be included in the final protocol.

Reference

1 J. Walker, "Verification of the Biological and Toxin Weapons Convention: The UK's Practice Compliance Inspection," in J.B. Poole and R. Guthrie, eds., *Verification Yearbook 1994: Arms Control, Peacekeeping, and the Environment* (Brassey's UK, 1994), pp. 135–144.

Parameters and Procedures for Sampling and Analysis

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5

In the discussions over means to bolster confidence in compliance with the Biological Weapons Convention, sampling and analysis is a contentious issue. Although in some cases sampling and analysis may be helpful in clarifying ambiguities regarding BWC compliance, it can also jeopardize confidential information unrelated to the convention, particularly at industrial facilities. Sampling and analysis by itself rarely offers definitive proof of compliance or noncompliance, and thus constitutes only one element in what has been described as the "web of deterrence" capturing would-be proliferators of biological weapons.[1] For this reason, sampling and analysis should be viewed only as a complementary tool, best used when combined with other verification measures to provide an indication of compliance. Because of its deterrent value, sampling and analysis should be included as a component of any BWC inspection regime, but only with full understanding of its limitations, including potentially deleterious effects with respect to confidential commercial or national-security information.

Making reasonable judgments regarding the utility and effectiveness of sampling and analysis depends on improved understanding of the balance between political and technical issues. This chapter addresses some of the questions surrounding the parameters and procedures that might apply to sampling and analysis in an enhanced BWC regime. It does not purport to provide definitive answers to those questions, but seeks to illuminate some of the tough decisions that lie ahead.

The Problem of Timing

The major challenge to the utility of sampling and analysis is timing: whether violations can be caught before evidence of illicit activity is destroyed. Because of the dual-use nature of most biological production equipment, a BWC violator—unless he were extremely careless—could eliminate within hours all traces of the agent being produced. The experience of the United Nations Special Commission on Iraq (UNSCOM) suggests that elements associated with a biological agent production capability could be identified, but that a "smoking gun" of a magnitude to offer definitive proof of noncompliance would be very difficult to discover.

To optimize the utility of sampling and analysis, inspections should ideally occur by

surprise, and access to a suspect facility should be immediate and unfettered. Nevertheless, recent discussions within the Ad Hoc Group (AHG) in Geneva suggest that countries will insist on reviewing any allegation of noncompliance before approving a challenge inspection. The AHG will also have to work out detailed procedures for providing access to a facility, conducting on-site inspections, and protecting the rights of the inspected party. Managed-access negotiations could create delays that, unless overcome by technology or diplomacy, might allow proliferators to dispose of incriminating evidence and thereby diminish the utility of inspection measures currently under consideration, including sampling and analysis.

The parameters for sampling and analysis—meaning the conditions under which it would be conducted—depend entirely on the nature of the on-site activities this measure is intended to support. Two broad categories of investigations have been proposed: field investigations and facility inspections. The second category has been subdivided into proposals for routine and challenge inspections, or "visits." Whether both types of visit will be included in the final protocol currently being negotiated by the AHG remains a matter of debate. Although several participating countries doubt the value of "nonchallenge" visits, they will be covered here for purposes of discussion.

Nonchallenge Visits

Nonchallenge visits, variously referred to as routine, validation, or quasi-random visits, would periodically confirm the accuracy of data submitted by facilities that have been declared by BWC states parties as being involved in treaty-relevant activities. In many cases, there would be no need for sampling and analysis because of the nature of the trigger that prompted the declaration. Also, alternative sources of data, such as record audits, interviews, or visual inspection of production equipment, might suffice.

From a cost/benefit perspective, employing sampling and analysis during nonchallenge inspections would provide a small direct return compared with the potential for loss of commercial proprietary information (CPI). Consequently, sampling and analysis should be minimized during such visits, unless anomalies indicative of a possible treaty violation are detected by other means. If sampling and analysis is employed, the inspectors need to determine the level of intrusiveness required to validate the declaration. Ultimately, this decision boils down to a political judgment because national policymakers must decide the level of confidence in BWC compliance they require.

Given the sensitivity about protecting CPI and national-security information unrelated to the BWC, sampling and analysis conducted during a nonchallenge inspection should generally be performed on site. Off-site analysis should be very rare indeed, and should occur only with the authorization of the inspected facility—unless it is needed to resolve a compelling suspicion of noncompliance. In such cases, legitimate commercial facilities would probably agree to additional testing to prove their innocence. Because the vast majority of routine visits will not uncover treaty violations, inspectors should avoid inconveniencing the inspected facility and disrupting its daily activities by insisting on sampling and analysis as a matter of course.[2]

Challenge Inspections

If unresolved discrepancies or other suspicions arise during a routine visit, the option should exist to shift the inspection into the challenge mode. Designating a specific threshold that would trigger this transition is difficult because the criteria are ultimately subjective. For this reason, the AHG might try to negotiate explicit procedures for how such a decision would be made. If the inspection-team leaders have the sole authority to make the decision, they might be accused of exploiting the challenge-inspection option for

frivolous or abusive purposes in a situation that is likely to be highly charged politically. Yet, because pursuing a suspected BWC violation may require expeditious action, presenting the observed anomalies to a multinational panel for review might take too long to coordinate. One solution might be to key the intrusiveness of the challenge inspection to the proliferation potential of the suspected violation. Although the inspected site would probably retain the right to refuse a challenge request, it would create suspicions by doing so.

Most challenge inspections will be initiated at the request of a state party. Such inspections would take place on short notice to resolve concerns raised by anomalies found during a routine inspection or other allegations of noncompliance (illicit production of biological or toxin agents, alleged use, or unusual outbreaks of disease). Once a challenge visit is initiated, an investigation team would seek evidence to prove or disprove the allegations. Sampling and analysis is a tool that could be of value, if exercised as necessary. In the current climate of the AHG negotiations, however, sampling and analysis is not likely to be endorsed as a measure that inspectors can employ routinely or automatically. Instead, it will probably be treated as an exceptional measure whose use is warranted only by serious, unresolved compliance concerns.

Most procedures associated with challenge inspections will almost certainly be conducted under "managed access," that is, subject to negotiation between the inspection team and the inspected party. Thus, even if the inspectors want to sample, under managed-access principles the inspected party will have the right to refuse. The inspectors will report such refusals, however, and the inspected party will be expected to offer a reasonable alternative means of answering the specific concern that sampling was meant to address.

Challenge inspections may occur at either declared or undeclared facilities. At a declared facility, the inspectors will be familiar with the production equipment and processes on site and will have identified specific areas of interest. At an undeclared facility, in contrast, the inspection team may need to inspect the entire site in considerable detail to address compliance concerns. Because of this difference, subcategories of challenge inspections might be designed with varying levels of intrusiveness. If commercial facilities knew that failure to comply with their obligation to file declarations would make them liable to a far more intrusive inspection, they would be more likely to comply with the declaration requirement. Of course, protections would have to be built into the system to ensure that BW proliferators—not just poor record-keepers—are the primary target.

Challenge-inspection requests based on vague suspicions of noncompliance are not likely to be approved. Instead, such requests must involve a formal accusation supported by sufficient evidence (intelligence, environmental samples, or epidemiological data) to stand up under international scrutiny. Once a challenge request has been lodged and accepted, the inspection is launched with the goal of obtaining evidence to confirm or disprove the specific allegations. Inspectors should not view a challenge inspection as a license to conduct an open-ended "fishing expedition." Rather, sampling and analysis should test for the presence or absence of specific pathogens or toxins in the context of the alleged violation. Limiting the scope of sampling to data specifically relevant to the challenge minimizes the risk of compromising CPI. Treating sampling and analysis as "a method of last resort" in the face of lingering ambiguities about compliance can further strengthen this protection.

Inspectors should arrive at the challenged facility with an authorized set of procedures that can be confirmed by the host personnel. This approach would help to prevent frivolous or abusive inspections or excessive sampling. An established set of procedures would also facilitate managed-access

negotiations between the inspection team and the inspected site, and could establish an audit trail through which the sample materials could be tracked.

If a challenge request is triggered by the discovery of anomalies during a routine inspection, the inspectors may wish to expand the scope of the investigation to include more intensive use of sampling and analysis. At the same time, the inspectors should limit the focus of their inquiry to investigating particular concerns.

Conduct of Sampling and Analysis

Sampling and analysis should be an option during all types of BWC investigations, but with a different priority and level of specific information sought. As discussed above, sampling should be a highly unusual occurrence during nonchallenge facility visits, if it occurs at all. In negotiating access to a facility during a challenge inspection, the inspection team should combine the purpose of the visit and the authorized inspection procedures with specific information about the site to produce an "inspection plan." Based on this plan, appropriate analytical equipment and reagents should be selected and brought on site.

At undeclared facilities, or those for which limited information exists, the inspectors need to survey the layout, production equipment, materials, and other objects from which samples might be collected. Declared facilities could be challenged based on suspicions of the diversion or misuse of declared equipment and materials, or because of the presence of undeclared equipment or materials thought to pose a risk to the BWC. In either case, the inspectors will have to develop a plan for collecting the evidence needed to fulfill their mission, and they likely will have to negotiate with facility or state-party representatives the location and means for taking a sample.

Once the preparations for sampling are completed, samples may be gathered from soil, water, air, culture media, air filters, and solid surfaces, and the relevant microorganisms or their DNA concentrated or multiplied to analytical levels with techniques such as the polymerase chain reaction (PCR). The material for analysis can then be separated and analyzed with a variety of methods (see the chapter by Morse in this volume). Other than sample theft, the analysis phase poses the greatest potential risk to CPI. Thus, analytical procedures should be selected not only on the basis of performance but also their ability to capture only those data required to support or refute specific allegations. Once the analysis is concluded, investigators should determine whether follow-up activities are warranted. The results should then be reported to the inspection team leader, including the methods of analysis, disposition of samples held, chain of custody, and disposal of sample remnants.

The inspection report offers another opportunity for the loss of CPI or national security information. With care, however, this risk can be minimized. If the purpose of the inspection is to prove or disprove the active production of a restricted microorganism, then the report need only state the bottom-line conclusions—a minimum "pass" or "fail"—without describing every detail of what was found inside the facility. At the same time, states parties should be allowed to draw their own compliance judgments by assessing the relevant aspects of the data. Supporting data should be stored for future reference—for example, sealed under lock and key at the inspected facility or filed at an off-site location under restricted access—but there is no need to report everything. If sampling and analysis confirms or reinforces concerns or suspicions that gave rise to the request to sample in the first place, more information will probably be required. The inspectors may

either request additional sampling immediately, or states parties may request another challenge visit during which the inspectors would conduct additional sampling.

Sampling Procedures

Uniform procedures for sample collection that can be shared with the inspected party would provide a useful basis for managed-access negotiations. In particular, facility managers must be able to discern whether anomalous activities are occurring during an inspection, such as the taking of unauthorized swab samples.

As part of any compliance protocol, states parties to the BWC should establish sampling standards related to factors such as biosafety level and quality assurance. These standards should be consistent with existing guidelines for biosafety and good manufacturing practice (GMP). While safety measures can be modified on a case-by-case basis, facilities suspected of developing or producing BW agents should be examined using maximum biosafety procedures. Prior to taking samples, the inspection team should resolve interim storage and chain-of-custody issues. Some samples, for example, may require storage under high containment.

Samples must be matched with specific analytical procedures to minimize the opportunity for diversion or excessive sampling and to ensure that the appropriate methods have been chosen. Indeed, various analytical procedures require samples of differing quantity or quality, while some sample preparation methods can modify the analyte so it is no longer suitable for other types of analysis. For example, an inactivated sample cannot be cultured for bioassay methods, and sterilization methods may inhibit the effectiveness of PCR.[3] For this reason, a sample should be divided before subjecting it to different types of analysis.

Sample size should be in keeping with the concentration or multiplication methods used, as well as the planned analytical procedure(s). The starting quantities needed for various concentration and analytical methods may range from a few tens of microorganisms for PCR to orders of magnitude more for less sensitive techniques. If the inspection team collects only as much material as is warranted by the analytical procedure(s), there is less opportunity for diversion of samples or sampling in excess of legitimate need. Moreover, although locations for the collection of samples are likely to be the subject of managed-access negotiation, to the extent possible they should be selected to minimize interfering with the operations of the inspected facility.

The number of samples taken also must be negotiated. At a minimum, the inspectors need to collect enough samples (together with controls) to ensure a statistically valid result. Moreover, the VEREX group concluded that the results of on-site sampling and analysis would be more reliable if several samples from the site yielded positive results when tested with two independent analytical techniques. Use of at least two such "orthogonal" methods would require a larger number of samples. For this reason, a maximum number of samples may have to be established, with the total increasing somewhat if the inspection team proceeds to a more intrusive phase of its investigation. The number of samples collected must also be tempered by fiscal realities because the cost of performing some of the more sophisticated analyses is considerable (see the chapter by Atlas in this volume). Given that states parties have already expressed a strong desire to keep costs down, the number of samples collected will likely be influenced, at least to some degree, by the BWC inspectorate's overall budget.

Control samples offer a point of reference to demonstrate what microorganisms natu-

rally occur in the facility's environment. By using control samples, the microbes associated with production at the plant can be distinguished from those endemic to the region in which the facility is located. Nevertheless, obtaining suitable control samples can be challenging. Ideally, controls should have been collected from the site before the inspected facility was built, but in most cases this is obviously not feasible. Instead, control samples may be collected from a nearby location that is similar enough to provide a reasonable surrogate. Even so, the validity of the control samples may be open to question in some cases.

Off-Site Analysis

Whereas off-site analysis is generally more accurate and reliable than on-site analysis, it also poses a greater risk for compromise of CPI. On-site analysis enables the inspected party to observe the analytical methods employed and to prevent diversion of the material, and hence is strongly preferred by industry. Technological advances continue to reduce the time needed to identify biological samples, facilitating the greater use of on-site analysis.[4] Because overly hasty analysis can jeopardize accuracy, it is necessary to strike a balance that allows enough time for reliable results but does not unduly burden the inspected party.

Off-site analysis is likely to be rare, but it should not be ruled out completely. When performed, off-site analysis should take place only at reference laboratories that have been accredited and validated according to procedures agreed on by all participating states. Such accredited laboratories require biosafety containment suites for work with suspected pathogens, as well as approved quality-assurance and control procedures. The international organization created to oversee implementation of the BWC protocol might be made responsible for the laboratory accreditation process.

According to one proposal for off-site analyses, samples together with appropriate controls should be sent to at least two accredited laboratories, and to a third in case of conflicting or ambiguous results.[5] As with on-site analysis, off-site analysis is best done with two orthogonal techniques. Moreover, the analysis should be "blind" with respect to the origin and identity of the sample to help safeguard CPI and ensure objectivity. In the event that an off-site laboratory is asked to analyze for novel agents, the inspectorate should not disclose the source of the sample but should provide guidance about suspected agents to facilitate identification.

To ensure continuous chain-of-custody of samples removed for off-site analysis, an authenticated audit trail must be maintained and all samples stored in secure containers with tamper-proof seals. Air transport of biological samples should follow current International Air Transport Association (IATA) regulations and recognize international import restrictions on pathogens. Upon arrival of the samples at the designated laboratory, technicians should immediately inspect the seals for signs of tampering and provide for separate, secure storage.

Other Approaches for Protecting CPI

Policy analysts have proposed a number of other approaches to minimize the risk that sampling could compromise CPI. For example, samples of microorganisms could be inactivated prior to analysis to render them nonviable. Inactivation would also enhance safety by diminishing the hazards associated with living, infectious agents. While inactivation of a sample would preclude the use of certain bioassays that require viable cultures, it would still allow for the use of powerful analytical techniques such as DNA probes and immunoassays.

Samples may also be subjected to treatment with "restriction" enzymes, which cut DNA strands at specific sequences of nucleotide bases. By fragmenting proprietary DNA sequences, restriction-enzyme treatment would enhance the protection of CPI afforded

by inactivation. This approach is not a panacea, however. In tandem, inactivation and digestion would restrict the amount of information that can be extracted from a sample, but unless the genetic sequences are fully broken down, they could in theory be reassembled and proprietary information compromised. Conversely, excessive digestion of the DNA could reduce the effectiveness of DNA probes and increase the risk of false negatives.

Live microorganisms should be taken off site only in the extreme case in which a violation is suspected and positive results have been obtained on site with at least two orthogonal analytical techniques. In this case, concern over protection of CPI is unlikely to apply, because any legitimate commercial facility would be seriously troubled by repeated indications that it is in violation of the BWC and would wish to resolve the issue as quickly as possible.

Another possible technical approach for safeguarding CPI would be to equip the analytical equipment with an electronic "filter" that displays data relevant to BWC compliance but screens out other types of information of strictly proprietary value. If such filtering devices can be developed, they should be tested and validated like all other analytical tools.

Finally, inspected parties should be allowed to insist on mandatory compliance with standing policies on biosafety and sterile procedures, such as the requirement that all visitors shower and wear a disposable coverall, surgical mask, head covering, and booties before entering sterile areas. If a member of the inspection team refuses to observe these established procedures, the inspected facility should have the right to request that individual's removal.

Analytical Equipment and Reagents

Analytical equipment used in investigations should be standardized. It should also be tested and calibrated before and after each inspection with standards accessible to all states parties to provide confidence that the analytical results are accurate. Efforts must also be taken to rule out tampering. Either side, if so inclined, could adjust the analytical equipment to collect data unrelated to the stated task. Just as there is a chain of custody and other measures to prevent tampering with samples, the same should apply to analytical equipment.

The issue of inspection equipment coming into contact with viable samples is one reason for the proposal that all microbial samples be inactivated and the DNA fragmented with restriction enzymes. It is impossible to provide 100% assurance that equipment has been completely cleaned of all traces of a proprietary microorganism. For this reason, inspected sites should be allowed to provide the analytical equipment (or at least those components that come in contact with the sample), provided that it conforms to the same rigorous standards applied to inspector-furnished equipment. In addition, either the inspectors or the inspected party should be allowed to provide reagents and other associated materials. Reagents should be standardized and validated, and the provider must be willing to supply control samples for analysis.

Portable analytical technology is advancing at a rapid pace.[6] As representatives of states parties deliberate in Geneva, they should continually evaluate new technologies that could enhance the inspection process. Such new devices may not only improve the ability to detect noncompliance, but they may achieve a better balance between bolstering confidence in BWC compliance and safeguarding proprietary information unrelated to the treaty. Once proven, such technologies should be integrated into the inspectorate's repertoire of analytical techniques.

Conclusions

Many issues must be resolved regarding the nature of sampling and analysis for BWC inspections. Two key questions stand out, however. First, does sampling and analysis make

a significant contribution to enhancing confidence in BWC compliance? Second, is that contribution worth the potential costs in terms of the risks that might be posed to interests unrelated to the BWC? Neither question can be answered at this time with a resounding "yes." While a number of procedures can be developed to facilitate the conduct of sampling and analysis, their contribution will be circumscribed by technical constraints and political realities, including procedures to protect proprietary information. As a result, sampling and analysis is not likely to become a central technique for assessing compliance with the BWC. Instead, it is more likely to serve a supporting function to other measures, and to be employed on occasions when ambiguities remain.

Central to the discussion of the costs and benefits of sampling and analysis is the need to protect CPI. A continuing dialogue between industry and government representatives on this issue is critical, particularly in light of ongoing technological developments. Such a dialogue will advance the negotiating process without putting important industry interests at undue risk.

Discussion

Strategies for safeguarding CPI. The discussion focused initially on technical approaches to sampling that would not jeopardize CPI. For example, one might utilize an assay that tests for specific bacterial toxin or capsular genes, and then treat the sample with a restriction enzyme that destroys CPI-related genetic material but not the target DNA sequences. With the proper PCR primer, one would need only a sample of about 20 microliters from the production line to test for putative BW agents such as anthrax. Inspectors might carry automated, sealed test kits and perform all analysis on site, and the size of the PCR primer could be set to permit selective identification of the target gene(s).

It was pointed out that even if this technology becomes available, larger questions loom. When is it appropriate to take samples? And what does one do with the information? When investigating a case of alleged use, the purpose of the sampling and the meaning of the results seem clear. But when taking samples at a civilian industrial complex, there are greater concerns over the use of the analytical results. If a future inspectorate tests too often and without appropriate discretion, it may lose credibility, particularly if no "smoking guns" are found. In deference to the political climate, the inspectorate may end up taking samples only in rare instances.

Deterrence effect of sampling. It was noted that the deterrent effect of sampling and analysis would be most effective against a country that is still in the planning stages of a biological-warfare program. A determined proliferator that has already produced and weaponized biological agents will probably continue doing so regardless of deterrence mechanisms. One participant observed that the weaponization of BW agents often takes place at a location separate from the production facility. Finally, it was emphasized that only a uniform set of inspection procedures, equally applicable to all companies and all countries, will be accepted by the international community.

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Evasion Scenarios and Countermeasures

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6

What is the potential ability of a Biological Weapons Convention (BWC) violator to evade detection under a sampling and analysis regime? If an illicit production facility could employ genetic-engineering techniques to disguise the BW agents it produced, or if it could remove *all* traces of BW agents by cleaning up prior to an inspection, sampling and analysis could be rendered less effective—although the effort to foil sampling and analysis might itself appear suspicious. This chapter examines possible evasion measures that might be employed by a proliferator, as well as countermeasures that could be taken by the BWC inspectorate.

Evasion scenarios related to *sampling* during an inspection are possible, but they will not be addressed here in detail. For example, if an employee of the inspected facility were allowed to perform the sampling, he might seek to alter the samples covertly in a manner designed to interfere with the analysis. One could ensure this had not happened, however, by taking a portion of the sample and adding a standard microorganism to ensure that the analytical technique could still detect it. While this procedure would require some elaboration to satisfy industry concerns, it would significantly reduce the possibility of evasion during sampling.

Other possible evasion scenarios would seek to undermine the effectiveness of various analytical procedures. Two types of scenarios are addressed here:

- ***Evasion scenarios based on molecular biology.*** These measures have a long lead time, from months to years, because they involve the genetic engineering of microorganisms, a task that is labor-intensive and requires sophisticated expertise.
- ***Evasion scenarios based on cleaning up a BW facility to remove all traces of agents in anticipation of an inspection.*** These measures have a short lead time, from as little as a few hours to a few days, depending on the facility.

Evasion Scenarios and Countermeasures Based on Molecular Biology

To understand how the DNA, or genetic material, of a microbial pathogen could be altered to evade detection, let's examine the flow of information from the DNA present in a cell to the synthesis of other cellular components.

The four-letter code in the DNA molecule is translated through an intermediary molecule known as "messenger RNA" (mRNA) into the 20 amino-acid code of proteins, the structural and functional building-blocks of all living cells.

The DNA molecule contains a sequence made up of four different "letters" or nucleotide bases: thymidine (T), cytosine (C), adenine (A), and guanine (G). The process of translating the sequences of DNA bases into the sequence of amino acids in a protein involves first creating a complementary mRNA transcript. This molecule has a similar four-letter code to that of DNA, except that the base thymidine (T) is replaced by the base uracil (U). DNA and mRNA are therefore identical in information content. Groups of three mRNA bases, known as "codons," specify different amino acids, which are linked together like a string of beads to form a protein.

The relationship between the codons and the amino acids is known as the "genetic code." For example, the three-letter mRNA codon **UUC** specifies the amino acid phenylalanine, whereas the codon **CGA** specifies the amino acid arginine. Because there are four mRNA bases, there are $4^3 = 64$ potential three-letter codons, or more than enough to code for the 20 amino acids found in proteins.

In fact, the amino acids (with one exception) are specified by more than one codon, so the genetic code is said to be "degenerate." For example, the mRNA codons **CGA** and **AGG** both specify the amino acid arginine. Some amino acids are specified by as many as six codons. Because a typical protein consists of 300 to 400 amino acids, the DNA sequence that directs the synthesis of a typical protein is about 1,000 bases in length, and is called a "gene." The full complement of DNA in the cell (i.e., the full set of genes) is known as the "genome."

The nonprotein components of the cell (such as sugars, fatty acids, neurotransmitters, and steroid hormones) are synthesized in sequences of biochemical reactions catalyzed by enzymes, which are also proteins. Thus, all parts of the cell are ultimately specified by the information contained in the base sequence of the genomic DNA.

The flow of genetic information in the cell has the following implications for biological sampling and analysis:

- To evade detection with a DNA probe, the genomic DNA must be altered.
- To evade immunoassays targeted at microbial proteins, the proteins must be altered. Yet to alter these proteins, the genomic DNA (or RNA in the case of some viruses) must be modified, because it directs the synthesis of all cellular proteins through the genetic code.
- To change the amounts and types of nonprotein components to evade detection by chemical analysis methods, the enzymes responsible for their synthesis must be altered, eliminated, or new enzymes added. Again, the genomic DNA must be modified because it directs the synthesis of enzymes through the genetic code.

It is clear that *all* inheritable alterations of microorganisms designed to evade detection must be made at the DNA level. How could molecular biologists make such alterations while retaining a viable and virulent BW microorganism? At least four kinds of DNA-level alterations might be possible for evasion purposes:

- Remove or inactivate the gene for a nonessential protein that is targeted by immunoassay, so that the entire protein is eliminated;
- Alter the DNA sequence of a gene so that it codes for the same or slightly altered protein in which the gene sequence cannot be detected by DNA probes;
- Insert a single gene for a protein toxin into the genome of a nonpathogenic microorganism to transform it into a pathogenic agent;
- Insert several genes for enzymes into a nonpathogenic microorganism to give it the capability to produce a nonprotein toxin, transforming it into a pathogenic agent.

How each of these strategies may be carried out, as well as possible countermeasures to each strategy, are discussed below.

Remove gene for a marker protein. The removal of a gene for a microbial protein would make it possible to evade detection by antibodies targeted against that protein, or by DNA probes targeted against the corresponding gene. This strategy is clearly restricted to proteins that are not essential to the survival or pathogenicity of the microorganism. Moreover, DNA-probe detection of a microbial gene could be evaded only if the DNA sequence against which the probe is targeted has been eliminated. The countermeasure is simple: employ immunoassays directed to species-specific proteins that are essential to the survival or pathogenicity of the BW microorganism, of which there are many.

Alter DNA sequence. A second evasion strategy would involve altering the DNA sequence of a gene to code for the same or a slightly altered protein that still retains its biological activity. This task could be accomplished by using degenerate codons that specify the same amino acid in the protein, or to substitute codons for other amino acids in regions of the protein that are not critical for its function. For example, if a DNA probe can recognize the DNA sequence GGATTGCCG**TTC**GTTAATC, which contains the sequence (in bold) whose mRNA transcript codes for the amino acid phenylalanine, one DNA base could be substituted and the sequence changed to TTT, whose mRNA transcript also codes for phenylalanine. As a result, even though the microbial DNA sequence has been altered, its mRNA transcript still specifies the same amino acid in the corresponding protein. Several DNA bases within a short sequence of the microbial DNA would have to be modified in this manner to make the sequence unrecognizable to a standard DNA probe.

More generally, the genetic engineering of microbial pathogens to evade detection would be difficult for a number of reasons. For a single DNA probe not to recognize its target sequence, at least one in every 10 to 15 bases would have to be altered. Thus, for a short probe consisting of about 30 bases, two to three substitutions in the corresponding microbial gene would be required to evade detection.

DNA sequence changes designed to evade detection by a DNA probe while leaving the corresponding protein unchanged would still not prevent detection of the protein by immunoassay. To evade immunological detection, the three-dimensional shape of the protein would have to be altered. A considerable amount of experimentation would be necessary to determine which changes in the DNA sequence alter the protein's shape to make it unrecognizable to the immunoassay yet are permissible for the retention of its functional activity. Thus, to evade both DNA-based and protein-based (immunoassay) detection, many carefully selected DNA alterations have to be made. Additionally, alterations may be necessary in DNA sequences from both the genes that encode the proteins targeted by immunoassay and other areas of the genome that may only be targeted by DNA probes. These alterations are hardly trivial to make, but if made can still be countered effectively.

Possible countermeasures to DNA sequence alteration are to prepare DNA probes for multiple gene targets, even though only a few probes would be needed to identify a microorganism with an adequate level of specificity. Having a large repertoire of DNA probes available would increase the difficulty of genetically modifying BW agents to evade detection by *all* available probes.

Another countermeasure would be to utilize DNA probes under lower stringency conditions, meaning that they can recognize target sequences in the microbial DNA that have slightly more than one-in-ten base substitutions. Because reduced stringency would increase the potential for false positives, this countermeasure would be unacceptable except as an indicator of the need for further evidence. However, because stringency is a

continuous variable, it may be possible to find analytical conditions under which false positives are minimized and the detection of codon alterations is still possible.

Add a new gene. Adding a single new gene to a normally *nonpathogenic* microorganism to transform it into a pathogenic agent is the strategy that would probably be easiest to implement and the one with the greatest potential for success. A gene for a protein toxin, for example, could be inserted into a normally nonpathogenic microorganism such as *E. coli*, or a weak pathogen that is not on the list of agents for which identification methods have been developed. Accordingly, this recombinant microorganism would not be identified as a BW agent even by a battery of standard DNA probes or immunoassays unless the assays target the toxin gene itself. To insert a gene for a protein toxin into a microbial genome is a routine task for recombinant DNA technology. Furthermore, the inserted toxin gene could not be identified if the DNA sequence coding for the toxin were modified to evade DNA probes directed specifically against the standard sequence. Making base substitutions in the toxin gene that preserve its toxicity yet evade immunoassay detection would not be prohibitively difficult.

Nevertheless, the amounts of a nonliving toxin required for military purposes are orders of magnitude greater than for infectious agents, which can multiply within the host after infection. In this regard, toxins are more like man-made chemical-warfare agents than infectious agents, and are also covered by the Chemical Weapons Convention (CWC). The difference in effective dose between toxins and microbial agents renders toxin production, storage, and weaponization much more vulnerable to detection, so that less sensitive analytical techniques might be acceptable.

As a countermeasure against the insertion of toxin genes into nonpathogenic microorganisms, the BWC inspectorate might develop sensitive immunoassays against all known protein toxins, as well as a battery of DNA probes against each known toxin gene. Such probes should also be capable of detecting toxin genes with substituted bases. Testing for the full set of base substitutions should be possible using the new DNA microchip technologies that employ short DNA probes of 15 bases in length (that is, only five codons). For appropriately chosen codon sets, typically only 100 different DNA probes would cover all possible base substitutions.

Of course, the utilization of new methodologies may raise additional evasion scenarios and countermeasures that need to be thought through. To avoid suspicion, however, the violator would have to be able to make a convincing case that his use of the supposed nonpathogenic microorganism is for a credible peaceful purpose. The weaponization of a new, genetically engineered BW agent would also require extensive study and field testing.

Add multiple genes. Adding several new genes to a nonpathogenic organism is a considerably more difficult task than adding a single protein-toxin gene. For example, a BWC violator might seek to insert into a microbial genome a set of genes coding for enzymes that catalyze the multiple chemical steps required for the synthesis of a nonprotein toxin (e.g., a small molecule such as saxitoxin). This task would require a good understanding of the biochemical pathways involved in toxin synthesis and how those pathways can be integrated into and not interfere with the existing metabolic pathways of the cell. Such detailed information is not currently available for most microorganisms of interest.

In addition, regulating the amounts of enzyme synthesized from each inserted gene would require very sophisticated genetic engineering. This capability is at the cutting edge of molecular biotechnology and would require a great deal of original research with no guarantee of success. In any case, immunoassays and/or chemical analyses for nonprotein toxins will likely be developed and could serve as effective countermeasures. (It is much harder to modify a nonprotein toxin

than a protein toxin while retaining its functional activity.) DNA probes and immunoassays could also be developed for key enzymes in the biosynthetic pathways.

To make an entirely new BW agent by converting a nonpathogenic organism into a pathogen would be even more problematic. Because the physiology of pathogenesis is complex and not well understood, this scenario seems unlikely. Moreover, a newly created pathogen would require exhaustive study from the ground up, because its properties and biological effects could not be predicted.

The various molecular-biology-based evasion strategies are summarized in Table 6-1.

Evasion Scenarios Based on Cleanup

Could a violator of the BWC evade detection by cleaning up an illicit production facility after receiving notice of an on-site inspection? There are two components to this question:

- Is there enough time to clean up a facility between notification of an inspection and the arrival of the inspection team at the site?
- Would BW agents or identifiable residues remain after the clean-up?

While few controlled experiments have addressed these issues, current industry practice can provide some partial answers. A number of parameters can influence the effectiveness of clean-up activities, including the type of BW agent produced, the nature of the target for the analytical method, the degree of biocontainment, and other particulars of the facility. These factors are discussed briefly below.

Type of BW agent. For BW microorganisms that are human pathogens, care is usually taken to keep the agent contained to prevent contamination of the facility. For plant and animal pathogens, less care might

Table 6-1. Possible evasion strategies based on molecular biology.

Evasion strategy	Development problems	Possible countermeasures
Knock out a nonessential gene to evade detection with gene probes or immunoassay for the corresponding protein	Only feasible for nonessential genes; requires advanced genetic engineering methods; long and tedious	Utilize assays for essential genes and their products or conduct fatty-acid profiles
Substitute codons to evade detection with gene probes	A genetically modified pathogen would have uncertain viability and pathogenicity; requires advanced genetic engineering methods; long and tedious	Develop several DNA probes and antibodies for multiple genes and their products; perform lower stringency testing with gene probes; do fatty-acid profiles
Insert gene for a protein toxin into the genome of a nonpathogenic microorganism	A genetically modified microorganism would have uncertain virulence, possible difficulties in weaponization	Utilize DNA probes to toxin genes (including lower stringency testing for modified toxin genes) plus immunoassays for protein toxins
Insert multiple genes for synthesis of nonprotein toxin into the genome of a nonpathogenic microorganism	Requires advanced genetic engineering methods and extensive basic research	Utilize DNA probes to genes for enzymes in biosynthetic pathway; do immunoassays and chemical analysis for nonprotein toxins

be taken to keep the facility uncontaminated because these agents pose no threat to human health. Even with human pathogens, immunization of workers against the infectious agent can make it possible to work with minimal biocontainment, as has occurred in Iraq and other proliferant countries in the past.

Nature of the target for analysis. The DNA molecule is extremely stable and more difficult to destroy than other cellular components; it can even be found in archeological or paleontological material of great age. Thus, DNA probe methods have the advantage that the target microbial DNA sequences may well remain intact after cleanup of a facility. Of particular interest is the observation that autoclaving (treatment with superheated, pressurized steam), the primary sterilization procedure in the biotechnology industry, may not destroy DNA. Indeed, there is an extensive literature on the use of PCR with degraded samples.[1] DNA molecules may also survive a variety of chemical sterilization treatments, including formalin, paraformaldehyde, or glutaraldehyde. Although DNA can be destroyed by harsh oxidizing agents, incineration, enzymatic digestion, intense ionizing radiation, or short-wave ultraviolet light, most of these treatments involve chemical or physical treatments that are either hazardous (e.g., radiation) or extremely expensive (e.g., enzymes) and hence would be difficult to apply to a large facility.

Degree of containment. While modern facilities operating under demanding good manufacturing practice (GMP) standards strive for perfect biocontainment, older facilities—particularly those designed for purposes requiring little containment—may become contaminated more easily. In the rush to clean up a facility in anticipation of an inspection, it is possible that some contaminated areas might be overlooked. Still, the inspectors would have to be lucky enough to take samples from a contaminated area. If special chemicals and enzymes (nucleases and proteases) were used to destroy telltale DNA and protein molecules, the discovery of these chemicals in a facility might give rise to suspicion of a BWC violation, depending on the declared use of the facility. This is an example of a situation in which the *possibility* of sampling and analysis might cause a violator to take an action that in itself would create suspicion.

Even in modern pharmaceutical facilities, containment can be imperfect. During a trial BWC inspection of a plant in Britain, DNA probes detected isolated microbial DNA outside a state-of-the-art fermenter, although no live microorganisms were found. Plant officials speculated that the detected DNA may have come from leaks in the steam-condensate system. Thus, evidence of BW microorganisms may sometimes be found because of imperfections in the production equipment. In addition, there is a reasonable probability of finding residues of past production in pipe joinings, which could be sampled if there were strong collateral evidence of a violation.

Inspectors could also look for evidence of a fresh cleanup, such as the installation of new air filters throughout the plant, the synchrony of plant operations (indicating that they were restarted at about the same time, following cleanup), the absence of seed stocks from storage facilities, and the contents of the waste stream (i.e., disinfectant load, absence of microbial residues). Finally, if inspectors were to find a high-containment facility in which the declared use did not indicate the need for such containment, that in itself would be cause for suspicion.

Particulars of the facility. A number of specific factors can influence the likelihood of contamination, the ability to clean up a facility, and the time required. Among them are the intended use for which the facility was constructed, its size, whether its location is isolated or in a populated area, the presence of high-containment systems and the expertise needed to operate them, and the availability of replacement equipment and parts.

Conclusions

Molecular biology provides a number of strategies by which a would-be violator of the BWC might seek to evade detection by DNA probe and immunoassay methods. Most of these evasion strategies would require considerable time and effort, however, and possible countermeasures are available. Devising batteries of tests directed at a variety of microbial DNA or protein targets would make evasion more difficult. Even though only a few tests in each battery might be used at a particular facility, a potential violator would need to counter most or all of them to ensure successful evasion, a daunting task.

The one possible exception is the insertion of a gene coding for a single protein toxin into a nonpathogenic microorganism. The genetic engineering methods required for this task are standard, and the inserted toxin gene could be extensively modified to prevent detection with DNA probes unless extensive countermeasures were undertaken. Even so, the protein toxin, once produced, could be detected by immunoassay. This example demonstrates the value of "orthogonal" analytic methods: one type of assay could be evaded but not the other. Indeed, no matter what evasion strategy is implemented, virulence testing in animals and plants is a powerful last resort.

The alteration of families of nonprotein molecules, such as fatty acids, would require considerable genetic engineering, and the significant changes needed to avoid identification of a BW microorganism by chemical analysis of amino-acid profiles would probably be lethal to the cell. Families of nonprotein molecules, therefore, are appealing targets for analysis. The drawback of standard chemical analytical methods is that they do not approach the sensitivity of DNA probe analysis, or even of immunoassay.

For all these reasons, attempts to use molecular biology to modify microbial pathogens and toxins genetically so as to evade detection will probably not take place in the near future. Facility clean-up is a more likely evasion scenario, at least in the near-term. Modern high-containment facilities can be thoroughly cleaned in a matter of hours (roughly one hour with clean-in-place systems, eight hours by hand), or less than the time between the notification of an inspection and the arrival of the inspection team at the site. However, most other facilities of interest do not have advanced containment and self-cleaning systems and hence would take a few days to clean. In the rush to clean up a plant before the inspectors arrive, a cheater could make mistakes and leave identifiable residues of BW microorganisms. The probability of identifying such residues will be much higher if there is some idea of which microorganisms to look for. Nevertheless, the mere possibility of sampling and analysis could deter potential violators by making illicit production risky or expensive, or by necessitating the use of harsh clean-up measures that would cast suspicion on the facility. This deterrent effect is likely to be the chief value of sampling and analysis in a BWC compliance regime.

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Discussion

Evasion strategies. An industry participant noted that although the paper covered the most obvious evasion strategies, it is difficult to dismiss the possibility that other, more effective strategies might exist in the future, given the diversity that biology provides and technical ingenuity. One should also consider the effort that a determined violator of the BWC could devote to evasion research and development if biologi-

cal warfare were a key element of that country's offensive military strategy.

Another participant asked about the level of molecular biology sophistication in BW proliferant states. It was noted that Iraq has some rather competent genetic engineers and a few biotechnology institutes, including one that is developing transgenic fish, but because of the trade embargo it has been unable to update its equipment and obtain needed materials. Nevertheless, participants agreed that given the rapid pace of advances in detection technologies, a potential cheater would be more likely to adopt a low-technology evasion method rather than a high-technology approach. For example, rather than employing advanced genetic-engineering techniques to develop a modified BW agent that leaves no signatures, a proliferator would be more likely to clean up after illicit activities.

An industry representative noted that the Klotz paper had overlooked a more likely evasion scenario raised in the Geneva debate. Instead of cleaning up production facilities or developing genetically modified microorganisms, a violator could simply declare that it possessed small quantities of virtually every microorganism or toxin of potential BW use, but for nonprohibited purposes. This strategy would render useless all of the extremely sensitive analytical techniques because the tests would always be positive under such circumstances, yet would provide no indication of the quantities of agents produced.

References

1 Federation of American Scientists, "New Approaches to Microorganism Identification," November 1995.

Costs and Benefits of Sampling and Analysis

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7

The Ad Hoc Group in Geneva is considering various types of on-site inspections, including routine and challenge visits, as part of a compliance-monitoring regime for the BWC. Such inspections would involve varying degrees of intrusiveness and cost, depending on which of the proposals under consideration are eventually adopted. For example, inspections might be triggered as a result of the alleged use of biological weapons or a suspicious outbreak of disease possibly related to such use, allegations of BW development or production, anomalies uncovered by other monitoring measures, or as a routine practice to confirm the accuracy of declarations submitted under the BWC protocol. Each of these types of inspections could involve sampling and analysis procedures.

Although it is almost certain that sampling and analysis will play some role in investigations of alleged use, discussions in the Ad Hoc Group on its application to on-site inspections of biological facilities have been contentious, and there is as yet no agreement on whether the potential costs outweigh the benefits. Proponents argue that the capability to detect microbial pathogens and toxins that could be used as BW agents is critical to ensure compliance with the BWC and would help deter the development, production, and use of biological weapons. But opponents counter that sampling and analysis is unlikely to detect biological weapons because they could be easily hidden or masked by the presence of microorganisms that occur naturally or are employed for peaceful industrial purposes. Many US federal agencies, such as the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC), routinely culture deadly pathogens for research activities aimed at developing treatments for human infectious diseases. Furthermore, many of the same microorganisms that can serve as biological weapons are cultivated by the pharmaceutical industry for the production of protective vaccines. Industry representatives also argue that sampling and analysis poses a high risk of loss of commercial proprietary information (CPI), including the compromise of specific strains of microorganisms that are highly valued for their biotechnological applications.

Although the UNSCOM experience points to the limitations of on-site inspections, in other cases inspections have uncovered biological agents that clearly were intended for use as weapons. For example, after the

attack with a chemical weapon on the Tokyo subway in March 1995, Japanese police found cultures of microbial pathogens at the facilities of Aum Shinrikyo, the suspected terrorist group. Because there was no legitimate purpose for having such cultures, the logical conclusion is that they were intended for use as biological weapons. Thus, in some cases on-site inspections can uncover conclusive evidence of illicit activities. The history of known BW development programs points to the most likely microorganisms and toxins for which to screen, and human intelligence may provide tips on where and when to sample.

This chapter examines the costs and benefits of various types of inspections that might involve sampling and analysis, in an attempt to identify an appropriate balance.

The detection of illicit BW agents would significantly benefit international security by enhancing compliance with the BWC and helping to prevent the enormous suffering and costs that would result from the actual use of biological weapons in an act of war or terrorism. Indeed, the deliberate release of a BW agent such as anthrax over a major city could inflict millions of casualties and cost billions of dollars to contain, manage, and remediate. Moreover, if the BWC compliance protocol provides for the investigation of unusual outbreaks of infectious disease, it would offer extensive benefits to international public health.

On the other side of the coin, inspections that fail to detect biological weapons properly, either because of false-negative or false-positive results, could be very costly. In assessing these costs, it is necessary to consider the need to protect the participating countries against the loss of their natural biodiversity resources, industrial secrets, and intellectual property rights. If sampling and analysis proves ineffective at detecting biological weapons and has little if any deterrent effect on illicit BW development or utilization, the associated costs would be unacceptable. There is also some risk that sampling and analysis could inadvertently spread pathogenic microorganisms.

Conducting sampling and analysis in a manner that maximizes its benefits and minimizes its costs has implications for the design of the inspection regime. Methods that provide the greatest confidence for detecting BW agents are likely to be the most intrusive and hence pose the greatest risks of compromising CPI. Conversely, making on-site inspections less intrusive by limiting sampling opportunities would reduce the costs of the regime to industry but would also weaken confidence in BWC compliance. A monitoring regime without sufficiently intrusive inspections could create false-confidence in treaty compliance and hence might be worse than no regime at all.

Nonchallenge Inspections

Nonchallenge inspections may be useful politically as a means of enhancing transparency and increasing confidence that activities underway at a biological development or production site are consistent with its declared purpose. Such inspections would be relatively easy to conduct in a cost-effective manner and would not compromise CPI if they did not require extensive use of sampling and analysis, and if access was carefully managed by the facility being inspected. For example, an audit of the balance between input of raw materials and output of finished products would give a strong indication whether a facility is doing what it claims.

In the context of nonchallenge inspections, the sampling and analysis of final products could confirm that the facility is engaged in a legitimate activity. If inspectors sampled only the final product, there would be no risk of loss of proprietary microorganisms. A visual inspection conducted as part of a routine visit would also reveal the presence of equipment that is not consistent with declared activities. Discrepancies revealed during a nonchallenge inspection could form the basis for a subsequent challenge inspection, in which more extensive sampling and analysis would serve to confirm or ease suspicions of treaty-prohibited activities.

Field Investigations

In contrast to nonchallenge inspections of facilities, field investigations of unusual disease outbreaks require extensive sampling and analysis. The specific tests employed depend on the suspected target organism(s), deduced from disease symptoms or pathology in humans, animals, or plants, or from background epidemiological data and/or intelligence information. Analyses would be targeted at a relatively small list of pathogens and toxins known to have been considered for use in BW development programs.

The results of such investigations may be ambiguous, however, because microbial pathogens are widely distributed in nature and there are many newly emerging or reemerging infectious diseases. Without adequate epidemiological background information, it would not be possible to assess with a high degree of confidence whether a biological agent originates from BW-related activities or from natural sources. Today, however, an adequate global surveillance network does not exist that is capable of generating the data needed to distinguish natural outbreaks of infectious disease from those resulting from the deliberate use or accidental release of BW agents.

An improved capability for global epidemiological surveillance would improve world public health and economic productivity, as well as international security, but creating the necessary infrastructure for investigating unusual disease outbreaks will be costly. The World Health Organization (WHO) has requested a $7 million increase in its annual budget to establish an early-warning system for epidemics, and the CDC has proposed an annual budget allocation of $125 million to address emerging infections. To the extent that inspections for biological weapons can be coupled with the public-health activities of the WHO and national agencies such as the CDC, the costs of field investigations would be reduced and the benefits to human well-being enhanced.

For example, when the first outbreak of Legionnaires' disease was recognized in 1976 during an American Legion convention in Philadelphia, there was early speculation that the disease epidemic was the result of a terrorist attack. It took years of research costing millions of dollars to establish that the disease was caused by a naturally occurring bacterium, *Legionella pneumophila*, which is widely distributed in water. Now that the causative microorganism, its reservoir, and mode of transmission are known, epidemiologists can rapidly diagnose outbreaks of Legionnaires' disease and determine the most likely causes of infection, so that further spread of the disease can be prevented.

Types of Samples

In carrying out sampling and analysis for potential detection of BW agents, great care must be taken to avoid exposure to samples containing deadly pathogens. Because biological samples are potentially capable of causing serious illness or death, all sampling and analyses must be conducted under conditions of high biocontainment (such as self-contained isolation suits) to ensure that inspectors and others are not inadvertently exposed. The cost of deploying such equipment is high, however, as has been demonstrated by the field investigation of the 1995 Ebola outbreak in Zaire. Samples containing viable microorganisms must also be shipped under high containment. Conducting analyses on site, using samples of killed microorganisms, would greatly lower the risks of exposure for inspectors and the general public to BW agents present in the samples.

When collecting samples during investigations of alleged production or use of biological or toxin weapons, one must take account of the environment in which the evidence is found. Different procedures are required when taking samples from the environment (air, water, soil), from fermentors in a production line, from human fluids or tissues, or from other media that may

contain pathogens or toxins. Specific sampling procedures are needed to obtain a high enough concentration of toxins, pathogens, or biochemicals to permit reliable identification. The amount of material needed to detect BW agents also depends on the efficiency of the extraction method and the sensitivity of the analytical procedure.

The greater the number of samples collected and analyzed, the greater the reliability of the results. However, collecting and analyzing a large number of samples increases costs and makes little difference if the samples are not of adequate quality to support the chosen analytical procedure. For example, because air generally contains low concentrations of microorganisms, large volumes of air must be filtered in order to collect enough particulate material for analysis. Air sampling is useful only when there is an actual BW aerosol present in the atmosphere, for example, during an accidental release or deliberate use of such agents. Similarly, in the case that biological agents were used to contaminate water supplies, the dilution effect would make it necessary to collect and concentrate hundreds of gallons prior to analysis.

The greatest likelihood of finding pathogens or toxins intended for use as BW agents would be inside production or storage vessels (or actual munitions), where high concentrations would be expected and only a few milliliters would provide a sufficient sample for analysis. But, US industry fears that the collection of samples from production vessels could result in the loss of valuable CPI.

The least cost to industry and potentially the greatest benefits are likely to result when samples are obtained from humans, animals, or plants suspected of having been exposed to BW agents. Because microbial pathogens multiply within the host in the course of infection and disease, they are concentrated in body fluids or tissues and can be readily sampled and analyzed. Such biomedical samples might be taken from ill or recovering individuals or obtained during autopsy. Medical epidemiologists have extensive experience in recovering pathogens from samples of tissue or body fluids because this approach is widely used for the definitive diagnosis of infectious diseases.

Analytical Techniques

The major analytical techniques each have specific sample requirements, strengths, limitations, and costs.

Bioassay techniques. Culturing viable microorganisms is the classic approach employed worldwide for the identification of pathogenic microorganisms and is the mainstay of field epidemiological investigations by organizations such as the CDC and the WHO. Hence, a great deal of comparative data are available, as well as experience in identifying viable cultures of microorganisms. The typical cost in a clinical laboratory in the United States to culture and identify a sample containing a microbial pathogen is $1.00 to $1.50, including reagents and labor. The cost per sample does not include the initial investment of approximately $50,000 to $100,000 for automated analytical equipment, but even taking this initial investment into account, the costs of culture methods are very low compared with those of other techniques. At the same time, bioassay methods entail the greatest risk of loss of CPI. Proprietary microbial strains containing genetic instructions for making a valuable industrial or pharmaceutical product may be worth many millions of dollars. The fear that these cultures could be compromised has led industry to resist the inclusion of bioassay methods in a BWC compliance regime.

Immunoassay techniques. In general, the cost of immunoassay is about 10 times greater than that of culture methods, although the exact cost varies depending on whether amplification procedures, such as the enzyme-linked immunosorbent assay (ELISA), are required. Immunoassays can confirm the presence or absence of suspected BW agents with a high degree of confidence. In addition, these assays can be used to identify killed

microorganisms whose DNA has been totally digested, eliminating the risk of losing proprietary industrial microorganisms or their genetic information. Immunological identification could therefore provide a good balance between effective identification of BW agents and protection of CPI.

Genetic analysis. PCR-amplified DNA probe analysis costs about 100 times more than culture-based identification methods. Nevertheless, the fact that this technique can be performed on killed microorganisms also reduces the risk that valuable CPI will be compromised. Even so, the risk remains of losing information contained in DNA sequences that have industrial value. Treatment with restriction enzymes to digest the microbial DNA prior to analysis could alleviate these objections by scrambling the genetic information, but it could also mask some target sequences in the microbial DNA and reduce the effectiveness of the technique for detecting BW agents. An alternative approach would be to incorporate the nucleotide base uracil during PCR and then use an enzyme (uracil N-glycosylase) to digest the amplified DNA after analysis, as is done in clinical laboratories to prevent contamination. This approach would provide more accurate analytical results while also protecting against loss of CPI.

The costs, strengths, and limitations of the three analytical methods are summarized in Table 7-1.

Conclusions

Devising sampling and analysis procedures that can enhance compliance with the BWC without jeopardizing CPI or national-security information has been an important focus of discussion within the Ad Hoc Group, as well as among various nongovernmental organizations. Regardless of whether inspections are a routine occurrence or take place only when there is compelling evidence of a violation, effective sampling and analysis procedures will be required. Such procedures must ensure the safety of the inspectors and the general public while providing assurance that BW activities can be detected.

While bioassay methods are likely to remain the mainstay for epidemiological investigations of unusual outbreaks of disease, sampling and analysis during on-site inspections of biological facilities should rely on immunological and genetic techniques, which can reduce the risk of compromising CPI by identifying dead microorganisms and denatured proteins. These safeguards are critical to promote cooperation between the inspection team and the inspected party. Preventing removal of viable cultures would also provide a measure of safety, reducing the risk that a live sample of BW agent could leak during transport or be mishandled during analysis and result in an accidental epidemic and fatalities.

In conclusion, restricting sampling and analysis to challenge inspections and field investigations of unusual disease outbreaks would limit costs and focus inspections in those areas in which they can have the greatest potential benefits, including (1) increasing transparency to enhance confidence in BWC compliance; (2) acting as a deterrent to biological weapons development, and (3) detecting any use of pathogens or toxins as weapons. By combining faster and more specific detection capabilities with the capacity to protect against loss of proprietary information, such analytical techniques promise to protect economic interests while enhancing global security.

Discussion

Limitations of cost-benefit assessment. One participant argued that a cost-benefit assessment is by nature an inaccurate tool for measuring the value of sampling and analysis. The problem is that the costs of BWC compliance monitoring are immediate, whereas the benefits are prospective and may not be readily apparent. Because the chief benefit of sampling is the nonoccurrence of a biological weapons attack or di-

Table 7-1. Costs, strengths, and limitations of three analytical methods.

Method	Strengths	Limitations	Estimated Cost
Bioassay	Standard procedure for epidemiological investigations; adequate data for comparison; provides material for additional confirmatory analyses.	Requires viable microbes and appropriate culture media and conditions; not all microbes can be cultured; does not discriminate from natural pathogens; requires days to weeks for results; increased risk to lab workers; risk of loss of CPI; cannot detect toxins.	$1–2/sample
Immunological	High specificity and sensitivity; does not require viable microbes; no need for high-level containment; rapid (minutes to hours); can detect toxins; low risk to lab workers; low risk of losing CPI; small amounts of target molecules if amplification procedures are used; no viable microbes needed.	Requires knowledge of appropriate target antigens; susceptible to interference by various agents in soil and tissues; highly target-specific.	$15–25/sample
Genetic	High specificity and sensitivity; rapid; does not require viable microbes; does not require high containment for safety; rapid (minutes to hours); preserved samples can be analyzed; low risk to lab workers.	Requires knowledge of appropriate target sequences; susceptible to interference by various agents in soil and tissues; very target-specific; requires purified target DNA or RNA; does not indicate viability of microbes; not applicable for detecting toxins; risk of losing CPI.	$100/sample

saster, its true value is difficult to quantify and may accordingly be underestimated. When an industry representative questioned the deterrence value of sampling, another participant pointed out that sampling could have determined the cause of the suspicious 1979 anthrax outbreak in the Soviet city of Sverdlovsk. Indeed, another participant noted that some 15 years after the Sverdlovsk epidemic, a team of epidemiologists was able to determine that it had been caused by an accidental release of anthrax spores from a military biological facility.

Workshop Findings and Recommendations

8

During the workshop, some general findings emerged that many participants could support, although consensus was not reached on every item. Findings receiving broad endorsement, as well as points of disagreement, are summarized below.

Utility of Sampling and Analysis

Sampling and analysis would be a valuable component of any legally binding BWC compliance protocol and, in conjunction with other measures, could be an effective deterrent of treaty violations. At the same time, sampling and analysis must be seen as part of a package of monitoring measures. Its appropriateness is scenario-dependent, based on an assessment of costs and benefits.

In isolation, sampling and analysis may not be definitive and could be misleading, and hence should not be relied upon as the sole source of evidence of BWC compliance or noncompliance. Because it is not possible to sample everywhere, a lack of evidence obtained by sampling could be exploited by a proliferator to claim a clean bill of health; conversely, contaminants could raise suspicions where none are warranted. To avoid unjust damage to reputations, the results of sampling and analysis should normally be corroborated with other types of evidence.

In crafting a BWC compliance regime, policymakers should strive for an approach to sampling and analysis that achieves a reasonable balance between costs and benefits. Even so, assessing the costs and benefits in any given situation is not a straightforward task. The costs of sampling in terms of potential losses of commercial-proprietary and national-security information can be calculated, yet the benefits of sampling in terms of deterrence of biological weapons acquisition and prevention of massive loss of life through avoidance of biological warfare are much more difficult to quantify. Moreover, the potential costs of sampling are immediate and real, whereas the benefits are long-term and hypothetical, yet no less important.

Sampling and Analysis Technologies

Sampling and analysis instruments should be developed that are portable, protect commercial proprietary information (CPI), and give accurate and consistent results. Such techniques must have both high sensitivity and

high specificity. In addition, sampling and analysis should be conducted in such a way as to avoid false positives and false negatives, for example, by testing multiple samples with at least two "orthogonal" analytical methods that are based on different scientific principles.

All assays must be rigorously validated in advance according to standards acceptable to industry and to government regulatory agencies such as the US Food and Drug Administration (FDA). At present, validated assays for the full range of biological and toxin warfare agents have not yet been developed. A database of microbial DNA sequences, such as the one being developed at the US Army Dugway Proving Ground, would also be a valuable tool for the genetic identification of samples.

To ensure proper analysis or to confirm controversial results, it may be necessary to conduct some analyses off site, and this option should not be ruled out. At the same time, off-site analysis could pose serious CPI and national-security concerns. To meet such concerns, it was suggested that in order to clarify serious anomalies that cannot be otherwise resolved, the inspection team should have the option of locking up a sample on site and bringing in additional analytical equipment for follow-up testing.

Safeguarding Commercial Proprietary Information

On-site inspections in general, and sampling and analysis in particular, must not compromise CPI or national-security information unrelated to the BWC. Industry representatives agreed that there are no CPI concerns associated with sampling the bulk finished product at the end of the production line. Three approaches to safeguarding CPI during on-site sampling and analysis activities are as follows:

(1) The use of "managed access" procedures, including giving the inspected facility the option to deny sampling and provide alternative means to address the inspectors' compliance concerns.

(2) Performing all analyses on site and limiting them to the identification of declared microbial agents or specific undeclared BW agents of concern.

(3) The proposal by the Federation of American Scientists to kill samples of proprietary microorganisms prior to analysis. Samples could also be treated with restriction enzymes to fragment proprietary DNA sequences.[1]

Possible Evasion Scenarios

Genetic manipulation of microorganisms to evade detection by genetic or immunological tests would be a nontrivial, labor- and expertise-intensive task. In general, countermeasures are available. While such evasion scenarios are unlikely at present, the future implementers of the BWC compliance regime must remain vigilant.

In theory, biological production equipment could be cleaned to eliminate all traces of biological or toxin warfare agents. In practice, however, cleaning is not always complete, particularly if the process is rushed. Participants agreed that "sloppiness is the sampler's best friend." The shorter the notification of an on-site inspection, the greater the probability that a BWC violator will make a mistake and leave telltale traces of illicit production that can be detected by sampling and analysis. Moreover, the presence of cleaning capabilities unwarranted by the declared use of the facility could raise questions about compliance.

Inspection Parameters and Procedures

The modalities of sampling and analysis vary under various proposed types of on-site inspections. For example, sampling and analysis could be conducted in either a "confirmatory" mode (to confirm production of a

declared item) or an "accusatory" mode (to pursue a suspected treaty violation). Nonchallenge (confirmatory) inspections probably would not normally require sampling from the process stream. At present, however, no consensus has been reached within the US government or the Ad Hoc Group on the definition, frequency, and duration of challenge and nonchallenge inspections, or even whether nonchallenge inspections should be included in the regime.

Several participants pointed out that many of the parameters characteristic of the Western biopharmaceutical industry, such as good manufacturing practice (GMP) standards, worker safety measures, and biocontainment systems, do not apply to dual-capable production facilities in suspected BW proliferant countries. For this reason, the BWC compliance protocol should not be designed from a Western perspective, but should cover the full range of production plants found in developing countries.

Some participants suggested that taking blood samples or saliva from workers at dual-capable biological production facilities and analyzing them for antibodies to undeclared biological and toxin agents would be a useful means of monitoring BWC compliance. (Saliva contains IgA antibodies and is the basis of a new test for the HIV/AIDS virus.) Others argued that unless a state party has already taken and stored blood samples, demanding that they be taken from plant workers for evidentiary purposes could pose intractable political and ethical problems. Because samples of saliva would obviously be less intrusive, this option is worthy of further investigation.

Recommendations

1. No list of putative biological and toxin agents should be taken as limiting the basic prohibitions of the BWC, which cover the development, production, transfer, and use of biological and toxin agents for warfare purposes, including genetically engineered microorganisms and toxins. For this reason, any list of agents developed to guide declarations should not be included in the protocol itself but in a subsidiary document that can be readily amended.
2. The development of sampling and analysis procedures in the context of a BWC compliance protocol should involve the active participation of all potentially affected industrial sectors and universities, as well as regulatory agencies such as the US Food and Drug Administration and its counterparts in other countries, with an eye toward optimizing effectiveness while minimizing intrusiveness.
3. Procedures for sampling and analysis in the BWC compliance protocol must be designed so that they can be applied uniformly to all states parties, including those in the industrialized West as well as those in the developing world. If the inspection procedures focus narrowly on Western-type facilities, an important segment of activities would not receive an adequate level of scrutiny.
4. The Ad Hoc Group should consider how to police the training and selection of international inspectors to minimize the risk of industrial espionage, and develop procedures for selecting and accrediting reference laboratories for off-site analysis.
5. Governments participating in the Ad Hoc Group should carry out trial inspections of industrial and military sites to clarify potential concerns over protection of CPI and national security information, including specific concerns related to sampling and analysis. To date, the United Kingdom, Canada, and the Netherlands have carried out a few trial inspections, but US industry has been reluctant to host them without a clearer sense of what the eventual compliance regime will entail. As the next step in pursuing trial inspections, government and industry representatives

should collaborate in developing a model set of inspection procedures that can be tested operationally and further refined.

Issues for Further Research

Workshop participants agreed that additional work is required in four areas:

1. The development of detailed parameters and procedures for sampling and analysis under various proposed types of inspections (e.g., challenge and nonchallenge facility inspections and field investigations). These scenarios would address the purpose of sampling, the type of evidence being sought, how the sampling and analysis would be carried out, and how the results would be reported.
2. Specification of detailed guidelines for sampling and analysis, including:
   - Development of analytical methods and procedures that protect CPI.
   - Procedures for validating various analytical techniques.
   - Implications of sampling for GMP standards.
   - Laws and regulations pertaining to the movement of samples off site.
   - Analysis of specific contexts in which environmental sampling (of soil, air, water, plants, and wild animals) would be of value.
   - Assessment of the feasibility and ethics of biomedical sampling in humans.
3. An analysis or simulation of managed-access procedures that addresses conflict resolution, what happens after access or sampling is denied, and how to prevent a BWC violator from using managed-access negotiation as a deliberate stalling tactic.
4. Further analysis of possible evasion scenarios.

Reference

1 Federation of American Scientists, "Sampling and Analysis of Proprietary Microorganisms While Protecting Confidential Proprietary Information," Pugwash Workshop on Strengthening the BWC, December 1995.

Author Biographies 9

Ronald M. Atlas is Professor of Biology at the University of Louisville, Kentucky. His research interest is in microbial ecology; he has pioneered methods for the bioremediation of oil spills and the molecular detection of pathogens in the environment using the polymerase chain reaction. Dr. Atlas is the author of some 200 scientific papers. He serves on the American Society of Microbiology's Task Force on Biological Weapons and represents the society on issues related to compliance monitoring of the Biological Weapons Convention.

Lynn C. Klotz is an independent consultant to the biotechnology industry specializing in technology-business strategy. He received his Ph.D. in chemistry from the University of California at San Diego and taught biochemistry at Harvard and Princeton Universities, where his research centered on DNA physical chemistry. Dr. Klotz was co-founder, Vice President, and Board Member of BioTechnica International and Managing Partner in the Devonshire Biotechnology Group. In addition to publishing more than 40 research papers and review articles and receiving two US patents, Dr. Klotz is co-author of a popular book, *The Gene Age: Genetic Engineering and the Next Industrial Revolution*. He is a member of the Federation of American Scientists' Working Group on Biological Weapons Verification.

Michael Moodie is President of the Chemical and Biological Arms Control Institute (CBACI) in Alexandria, Virginia. He has more than 20 years experience on international security issues, both in government and in the policy research community. During the Bush Administration, Mr. Moodie served as Assistant Director for Multilateral Affairs at the US Arms Control and Disarmament Agency (ACDA) where, among other issues, his bureau had lead responsibility for negotiating the Chemical Weapons Convention and for issues relating to the Biological Weapons Convention. Mr. Moodie is also an internationally recognized authority on regional arms control, with an extensive record of publications and presentations around the world.

Stephen S. Morse is Assistant Professor of Epidemiology and Director of the Program in Emerging Diseases at the Columbia University School of Public Health in New York City. In December 1996, he became a Program Manager at the Defense Advanced Research Projects Agency

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William L. Muth is Research Advisor at Lilly Research Laboratories in Indianapolis. He received a B.S. in bacteriology from Indiana University and a Ph.D. in microbiology and public health from Michigan State University. Dr. Muth has worked at Eli Lilly for 23 years on the fermentation of natural products and recombinant proteins, and has devoted 18 of those years exclusively to high-tech products of biotechnology.

Geoffrey W. Nagler is a Research Fellow at the Chemical and Biological Arms Control Institute and a Senior Analyst with EAI Corporation. He is a biologist and an expert on the implications of the Chemical and Biological Weapons Conventions for industry. Mr. Nagler has participated in several studies for the US government on the CWC and is an acknowledged authority on natural toxins and their coverage under arms control treaties.

Richard O. Spertzel is Chief of the Biology Section at the United Nations Special Commission on Iraq (UNSCOM). He received a V.M.D. from the School of Veterinary Medicine at the University of Pennsylvania and a Ph.D. in microbiology from the University of Notre Dame. Dr. Spertzel was on active duty with the US Army for 28 years, devoting most of his career to biological warfare and biomedical defense. His military assignments included 14 years with the US Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, Maryland. Dr. Spertzel retired from the Army in 1987 and subsequently provided consulting services on biomedical research, the assessment and prevention of terrorist use of biological and toxin weapons, and biowarfare defense. He joined UNSCOM in April 1994 and has since participated in more than 20 inspections in Iraq.

Jonathan B. Tucker directs the Chemical and Biological Weapons Nonproliferation Project at the Center for Nonproliferation Studies of the Monterey Institute of International Studies in California. He holds a B.S. in biology from Yale University and a Ph.D. in political science from the Massachusetts Institute of Technology, with a concentration in defense and arms control studies. In 1976–1979, he was a member of the Board of Editors of *Scientific American* magazine. During six years with the US government, Dr. Tucker worked as an arms control fellow at the Department of State, an analyst at the congressional Office of Technology Assessment, a foreign affairs specialist in chemical and biological arms control at the US Arms Control and Disarmament Agency, and a senior policy analyst on the staff of the Presidential Advisory Committee on Gulf War Veterans' Illnesses. He was a member of the US delegation to the Chemical Weapons Convention Preparatory Commission in The Hague in 1993–1995, and he served on an UNSCOM biological weapons inspection team in Iraq in February 1995.

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Glossary 11

A	adenine
ACDA	Arms Control and Disarmament Agency
AHG	Ad Hoc Group of BWC States Parties
BMVC	Baghdad Monitoring and Verification Center
BTW	biological and toxin warfare
BWC	Biological Weapons Convention
C	cytosine
CBM	confidence-building measure
CDC	Centers for Disease Control and Prevention
CGSR	Center for Global Security Research
CNS	Center for Nonproliferation Studies, Monterey
codon	group of three mRNA bases
CPI	commercial proprietary information
CWC	Chemical Weapons Convention
DARPA	Defense Advanced Research Projects Agency
ELISA	enzyme-linked immunosorbent assay
FAS	Federation of American Scientists
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared
G	guanine
GC/MS	gas chromatography/mass spectrometry
gene	DNA sequence that directs synthesis of typical protein
genome	full complement of DNA in cell
GLC	gas-liquid chromatography
GMP	good manufacturing practice
IATA	International Air Transport Association

LLNL	Lawrence Livermore National Laboratory
MIIS	Monterey Institute of International Studies
mRNA	messenger RNA
NIH	National Institutes of Health
NMR	nuclear magnetic resonance
OMV	ongoing monitoring and verification program in Iraq
PCR	polymerase chain reaction
PhRMA	Pharmaceutical Research and Manufacturers of America
ProMED	Program for Monitoring Emerging Diseases
RFLP	restriction-enzyme fragment length polymorphism
SOP	standard operating procedure
T	thymidine
U	uracil
UN	United Nations
UNSCOM	United Nations Special Commission on Iraq
USAMRIID	US Army Medical Research Institute of Infectious Diseases, Ft. Detrick
VEREX	Ad Hoc Group of Governmental Experts To Identify and Examine Potential Verification Measures from a Scientific and Technical Standpoint
WHO	World Health Organization

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